

1 **Wdr5, Brca1 and Bard1 link the DNA damage response to the** 2 **mesenchymal-to-epithelial transition during early reprogramming**

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15 16 **SUMMARY**

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18 Differentiated cells are epigenetically stable, but can be reprogrammed to pluripotency by
19 expression of the OSKM transcription factors. Despite significant effort, relatively little is known
20 about the cellular requirements for reprogramming and how they affect the properties of induced
21 pluripotent stem cells (iPSC). We have performed high-content screening with siRNAs targeting
22 300 chromatin-associated factors. We used colony features, such as size and shape, as well as
23 strength and homogeneity of marker gene expression to define five colony phenotypes in early
24 reprogramming. We identified transcriptional signatures associated with these phenotypes in a
25 secondary RNA sequencing screen. One of these phenotypes involves large colonies and an early
26 block of reprogramming. Double knockdown epistasis experiments of the genes involved, revealed
27 that Brca1, Bard1 and Wdr5 functionally interact and are required for both the DNA damage
28 response and the mesenchymal-to-epithelial transition (MET), linking these processes. Moreover,
29 the data provide a resource on the role of chromatin-associated factors in reprogramming and
30 underline colony morphology as an important high dimensional readout for reprogramming
31 quality.

32 33 **INTRODUCTION**

34
35 Somatic cells can be reprogrammed to pluripotency by artificial expression of four transcription
36 factors: Oct4, Sox2, Klf4 and c-Myc (OSKM) (Takahashi and Yamanaka, 2006). With varying
37 efficiency, iPSC cells can be derived from a wide variety of cell types and they can differentiate into
38 all cell lineages. Thus, they represent a promising resource for tissue regeneration and disease
39 modeling.

40
41 The earliest phase of reprogramming involves dramatic changes in metabolic and cellular
42 processes (Panopoulos et al., 2012; Polo et al., 2012) accompanied by an increase in cell
43 proliferation. Somatic genes are repressed (Maherali et al., 2007; Mikkelsen et al., 2007) and cells
44 undergo MET, a mesenchymal-to-epithelial transition (Li et al., 2010; Samavarchi-Tehrani et al.,
45 2010), leading to the expression of epithelial genes such as *E-Cadherin* (*Cdh1*) and *Epcam*, while
46 mesenchymal regulators (e.g. *Snai1/2*, *Zeb1/2*) are repressed (Samavarchi-Tehrani et al., 2010).

47 Subsequently, pluripotency genes carrying active histone marks at regulatory regions are activated
48 (Maherali et al., 2007; Mikkelsen et al., 2007; Polo et al., 2012). At this point, cells have not fully
49 acquired the pluripotency program. These partially reprogrammed intermediates are sometimes
50 referred to as pre-iPS cells (Silva et al., 2008). Late pluripotency markers and endogenous Nanog,
51 Oct4 and Sox2 are activated through a combination of promoter DNA-demethylation (Gao et al.,
52 2013; Meissner et al., 2008) and depletion of repressive histone mark H3K9me3 (Soufi et al., 2012;
53 Sridharan et al., 2013). The majority of the cells seem refractory to reprogramming or are trapped
54 in a partially reprogrammed state, and only a small percentage of cells will successfully progress
55 through all the stages (Polo et al., 2012).

56
57 A DNA damage response is important for reprogramming, as the p53 pathway prevents survival of
58 cells with substantial DNA damage (Marion et al., 2009). In agreement with this, DNA repair and
59 recombination proteins are required for reprogramming (Gonzalez et al., 2013; Hansson et al.,
60 2012). Additionally, senescence evokes a DNA damage response and it has been shown to be a
61 barrier for reprogramming (Utikal et al., 2009).

62
63 All these events reveal the importance of remodeling the transcriptional program and the
64 chromatin state during reprogramming. Several chromatin-associated proteins that facilitate or
65 block reprogramming have been identified by RNAi (Cacchiarelli et al., 2015; Qin et al., 2014). The
66 activities of the H3K9 methyl transferases Ehmt1/2, Suv39h1/2 and Setdb1 constitute roadblocks
67 of reprogramming (Soufi et al., 2012; Sridharan et al., 2013). In contrast, H3K9 demethylases such
68 as Kdm3a/b and Kdm4c facilitate reprogramming (Chen et al., 2013). In addition, both the
69 repressive Polycomb PRC2 complex (Onder et al., 2012) and the Trithorax SET-MLL methyl
70 transferase complexes act as facilitators. The H3K4 methylation mediated by the SET-MLL
71 complexes primes pluripotency enhancers for activity (Wang et al., 2016) and the absence of their
72 core component Wdr5 abrogates reprogramming (Ang et al., 2011).

73
74 Despite the progress that has been made in characterizing the molecular changes during
75 reprogramming, how these dynamic changes are orchestrated is still ill-understood. We have used
76 high-content screening to assess the role of ~300 chromatin-associated proteins in pre-iPS colony
77 phenotypes during early reprogramming. High-content analysis allows simultaneous measurement
78 of multiple morphological phenotypes. The combination of siRNA screening with high-content
79 microscopy can reveal new associations among pathways (Fischer et al., 2015; Sero and Bakal,
80 2017). A similar approach has previously been used to define new gene networks involved in the
81 final phase of iPS cells formation (Golipour et al., 2012). We measured over twenty colony-
82 phenotypes, including number of colonies, expression of pluripotency markers and other
83 morphological and textural features, after individual knockdown of 300 chromatin modifiers.
84 Selected hits from the primary screening were subjected to a transcriptome-based secondary
85 screen. We identify several chromatin-associated proteins that act together in the DNA damage
86 response and the MET during early reprogramming to pluripotency.

87

88 **RESULTS**

89
90 **High throughput analysis of the early phase of reprogramming**

91
92 Reprogramming is associated with major changes in cell morphology, in part due to the MET (Li et al., 2010). Thus, we asked whether chromatin-mediated changes would affect reprogramming efficiency, colony morphology and expression of pluripotency markers. Moreover, we wondered how chromatin-associated factors might work together, as revealed by their similarities in a high dimensional phenotypic space upon knockdown (Mulder et al., 2012; Wang et al., 2012). To define a set of relevant chromatin-associated factors for an siRNA screen (Fig. 1A), we used available expression data (Chantzoura et al., 2015) to select genes with robust expression MEFs or at least 4-fold upregulated expression in reprogramming cells. The custom siRNA library comprised 300 chromatin-associated factors, and for each target, three different siRNA molecules were pooled for transfections (Table S1).

101
102
103 We were specifically interested in the early phase of reprogramming, as chromatin is hypothesized to confer epigenetic stability to somatic cells. To test the function of the chromatin-associated genes in early reprogramming, we used a fast and efficient reprogramming system (Vidal et al., 2014), where colonies can be detected after 6 days of reprogramming (Fig. 1B, S1A). These colonies present characteristic round, symmetric morphologies and robust expression of early markers Cdh1, SSEA1 and Sall4, with expression of late markers such as Nanog and Esrrb appearing later (Fig. S1B-D). The specific staining of Cdh1 and Sall4, respectively at the cell surface and in the nucleus, strongly increased between days 3 and 6 (Fig. 1B, S1), representing a suitable readout for the early phase of reprogramming.

111
112
113 The expression of genes was knocked down using siRNAs in mouse embryonic fibroblasts (MEFs) infected with an inducible OSKM-cassette lentivirus. Reprogramming was induced with doxycycline (dox) for 6 days (Fig. S1A). The siRNA library consisted of six 96-well plates, with each plate containing seven non-targeting siRNA (nt) negative controls and three positive controls (siRNA targeting Trp53, Oct4, and c-Myc). The screen was performed in quadruplicate. After six days of reprogramming, samples were fixed, stained for Cdh1 and Sall4 and imaged using an automated high-content microscope. This allowed quantitation of morphology features such as colony size, symmetry and shape, marker intensities, but also texture features, which are a reflection of signal intensity patterns within colonies. After data processing and colony feature extraction, the data were z-score normalized per plate (Bakal et al., 2007) and subjected to further analysis (Fig. 1A).

124
125 To test the system, we disrupted reprogramming by knocking down the OSKM factors Oct4 (siOct4) and c-Myc (siMyc). We also knocked down Trp53 (siTrp53), which is expected to enhance reprogramming (Marion et al., 2009). siOct4 and siMyc colonies are flat, irregularly shaped and they show less intense Sall4 and Cdh1 expression compared to the control (Fig. 1C). Likewise, the number of colonies observed in siOct4 and siMyc in our z-score-ranked data was very low (Fig. 1D). The siTrp53 control showed a variable but positive effect on the number of colonies. These data confirm that colony morphology and pluripotency marker expression can be used as readout for a disruption in the early reprogramming network.

132
133

134 **High-content microscopy reveals five major phenotypes of colony formation**

135

136 The high-content analysis allowed us to measure not only the number of colonies, but also colony
137 features such as the intensity of early pluripotency markers (Sall4 and Cdh1), size, compactness
138 and symmetry, texture and many other morphology features (Table S2). These features constitute a
139 multidimensional phenotypic space for analysis across many conditions or perturbations (Boutros
140 et al., 2015) and the identification of functionally connected genes and processes (Mulder et al.,
141 2012; Wang et al., 2012).

142

143 We first defined the set of most discriminating features based on feature-to-feature pairwise
144 correlations (Supplemental Information; Table S3). Using hierarchical (Fig. S2) and K-means
145 clustering (Fig. 2A) we observed five main clusters that display different levels of pluripotency
146 markers, number of colonies, symmetry features (ratio width to length, roundness), STAR
147 morphology features, and textural features (SER, Harlick, Gabor). Cluster 1 knockdowns have few
148 colonies, in addition to low intensities for Sall4 and Cdh1, suggesting a major defect in
149 reprogramming. The majority of nt controls are in cluster 2, which shows a high number of small,
150 round and compact colonies and a robust expression of Cdh1 and Sall4 (Fig. 2A-B). Cluster 3 is
151 quite distinct with fewer, large colonies with low compactness features and detectable Sall4 and
152 Cdh1 expression (Fig. 2A, cf. Brca1 and Wdr5, Fig. 2B). Cluster 4 shows somewhat reduced Sall4
153 and Cdh1 expression and a reduction in some of the DAPI texture features, but is otherwise
154 relatively normal. Essentially all Oct and Myc controls clustered together in cluster 5, characterized
155 by substantially lower Sall4 and Cdh1 intensities, in addition to irregular, less round and less
156 compact colonies (cf. Ncor1, Fig. 2B).

157

158 To provide more insight in the nature of the imaging phenotypes, we compared all knockdowns to
159 each of the positive controls (Trp53, Myc and Oct4) by Pearson correlation, based on high-content
160 features. After ranking all knockdowns according to their combined correlation score
161 (Experimental Procedures), we selected 10 candidates from the top-ranking list (Table S4).
162 Additionally, the high-content data from known reprogramming facilitators present in our library
163 were used to train two independent machine-learning algorithms, in order to predict other
164 potential facilitators (Fig. 2C, Supplementary Information, Fig. S2, Table S4). This approach allowed
165 us to select additional candidates of high, intermediate and low-ranking prediction scores (Fig. 2C).
166 A total of 30 genes were selected for an orthogonal transcriptome screen (Fig. 2C, Table S4).

167

168 **A transcriptome-based secondary screening uncovers highly correlated phenotypes**

169

170 We hypothesized that the phenotypes observed by microscopy might be reflected in their
171 transcriptomes. Cells were transfected with siRNAs in triplicate and day 6 RNA samples were
172 subjected to CEL-Seq2-based RNA-sequencing (Hashimshony et al., 2016). In addition to the 30
173 knockdowns, we also sequenced a day-by-day reprogramming time-course of control cells (Fig.
174 3A).

175

176 We performed Principal Component Analysis (PCA) to the siRNA dataset for dimensionality
177 reduction. The pairwise correlations between all the transcriptomes were calculated based on the
178 top 200 transcripts associated with PC1 and PC2, and then clustered (Fig. 3B, left). We calculated
179 similar pairwise correlations for the microscopy data, and identified gene pairs that correlated in

180 both their colony phenotype and their transcriptome (Fig. 3C). The strongest correlations are
181 observed between the *Ncor1* - *Oct4* pair and a triplet consisting of *Wdr5*, *Brca1* and *Bard1*. *Ncor1*
182 was recently shown to physically interact with *Myc* and *Oct4* (Zhuang et al., 2018), but the
183 functional relationships between *Wdr5*, *Brca1* and *Bard1* were unknown. We performed siRNA
184 deconvolution experiments measuring the number of *Sall4*-positive colonies of three independent
185 siRNAs for *Wdr5*, *Brca1* and *Bard1* to exclude off-target effects. This analysis resulted in
186 phenotypes similar to the pooled siRNAs in at least two out of three siRNA sequences with the
187 same target (Fig. S3). In addition, high knockdown efficiencies of the *Brca1*, *Bard1* and *Wdr5* mRNA
188 targets were verified at day 3 of reprogramming (Fig. S3).

189
190 As reprogramming is a dynamic process, we wondered how cells progress towards the iPSC state in
191 each of the knockdown conditions. Notably, in PCA analysis, principal component 2 correlates
192 strongly with time ($r^2 = 0.81$; Fig. S4). To model the progression in each knockdown more
193 precisely, we fitted a polynomial function to the time points and projected all other data on the
194 time line by shortest distance (Fig. 3D, Experimental procedures). This distance reflects
195 transcriptome changes that are unrelated to normal progression of reprogramming. Most siRNA
196 knockdown transcriptomes, including the non-targeting (nt) and mock transfected controls have a
197 transcriptome that is in between day 5 and day 6 of reprogramming, reflecting a mild non-specific
198 effect of transfection. Silencing *p53* and *Hdac1* modestly speeds up reprogramming relative to nt
199 controls (Fig. 3D). Three other genes, notably *Wdr5*, *Brca1* and *Bard1*, show a strong delay in
200 reprogramming with a short distance to the time projection of control cells. si*Wdr5* cells were
201 comparable to normal cells between day 3 and 4, while si*Bard1* and si*Brca1* were between day 4
202 and 5 (Fig. 3D). We analyzed our time series data to relate the early block observed with si*Wdr5*,
203 si*Brca1* and si*Bard1* to known early reprogramming processes. The block is observed at the time of
204 a major decrease of mesenchymal gene expression and preceding the activation of epithelial
205 markers (Fig. 3E). For DNA repair and cell cycle genes there is an early wave of increased
206 expression followed by downregulation, whereas random genes are stably expressed over the time
207 course of reprogramming (Fig. S4). This time line raised the possibility that *Wdr5*, *Brca1*, *Bard1*
208 affect the repression of mesenchymal gene expression and the DNA damage response during early
209 reprogramming. Moreover, based on the phenotypic and molecular co-correlation data we
210 hypothesized that *Wdr5*, *Brca1* and *Bard1* functionally cooperate to control early stages of
211 reprogramming.

212 213 ***Brca1*, *Bard1* and *Wdr5* functionally interact during early reprogramming**

214
215 We asked whether *Wdr5*, *Bard1* and *Brca1* genes have similar expression dynamics during early
216 reprogramming. Interestingly, the three genes follow a similar RT-qPCR profile, peaking in
217 expression at day 3, and then slowly going down (Fig. 4A). To test the possibility of a functional
218 interaction between these genes, the effect of their respective double knockdowns was measured
219 and compared to the effect of the single knockdowns with regards to the number of pre-iPS
220 colonies formed. All three single knockdowns displayed a significant reduction in number of *Sall4*-
221 positive colonies, compared to the nt control (Fig. 4B). Therefore, the phenotypes of double and
222 single knockdowns were calculated as the *Sall4*-positive colony ratio compared to the control.
223 *Brca1-Bard1* double knockdown showed significantly more colonies than expected (Fig. 4C, left).
224 This result was anticipated, as *Brca1-Bard1* are well known physical interactors (Wu et al., 1996).
225 Similarly, for both the *Wdr5-Brca1* and the *Wdr5-Bard1* double knockdowns, we also observed

226 more colonies than expected, and this result was statistically significant for *Wdr5-Brca1* (Fig. 4C).
227 To test whether *Wdr5* is directly activating *Brca1* and *Bard1* gene expression, we determined the
228 *Brca1* and *Bard1* expression levels after *Wdr5* knockdown (Fig. 4D). Indeed, we find that this is the
229 case at day 3, but also find that in response to either *Bard1* or *Brca1* depletion, *Wdr5* expression
230 was decreased. Taken together, *Brca1*, *Bard1* and *Wdr5* are co-expressed, mutually depend on each
231 other, and interact functionally in reprogramming.

232

233 ***Wdr5*, *Bard1* and *Brca1* are functionally connected in the DNA damage response pathway**

234

235 *Brca1* and *Bard1* have a known function in double strand break DNA repair. If *Brca1* and *Bard1*
236 functionally interact with *Wdr5*, the prediction is that that all three knockdowns show an increase
237 in DNA damage. The phosphorylated form of the histone variant H2A.X (γ H2A.X) represents a
238 reliable biomarker for DNA damage, because it is an immediate response upon the presence of
239 double strand breaks (Sharma et al., 2012). Therefore, we employed FACS analysis to measure
240 γ H2A.X in the knockdowns (Fig. 5A-B). Reprogramming cells (nt control) showed a significant
241 decrease in DNA damage response as compared to non-reprogramming MEFs, in agreement with
242 literature showing that reprogramming resolves part of the DNA damage in somatic cells (Ocampo
243 et al., 2016). Importantly, *Wdr5* knockdown showed a significantly increased level of γ H2A.X
244 compared to the control (Fig. 5A). Nearly 90 % of the cells harbour γ H2A.X in *Wdr5* depleted cells
245 (Fig. 5B, bottom panel). As expected, si*Brca1* and si*Bard1* also showed a high percentage of γ H2A.X
246 positive cells (Fig. 5A, panels 2 and 3). To cross-validate our findings, we visualized γ H2A.X by
247 immunofluorescence. At day 3 of reprogramming, knockdown cells and controls were stained for
248 either Oct4 (Fig. S5) or SSEA1 (Fig. 5C), and γ H2A.X. In agreement with the results from the FACS
249 analysis, nt control transfected reprogramming cells showed a decrease in γ H2A.X compared to the
250 MEFs. Depletion of *Brca1*, *Bard1* or *Wdr5* impairs the DNA damage response in reprogramming
251 cells, resulting in more phosphorylated γ H2A.X (Fig. 5, left and right). It should be noted that few
252 cells or colonies show expression of SSEA1 in the si*Wdr5* cells, reflecting the early block of
253 reprogramming progression (Fig. 3D).

254

255 ***Wdr5*, *Brca1* and *Bard1* are required for MET and DNA repair gene expression**

256

257 Based on their timing of expression and the observed early block in reprogramming, we
258 hypothesized that *Wdr5*, *Brca1* and *Bard1* also affect the MET. To test this hypothesis and to gain
259 more insight into the *Wdr5*, *Brca1* and *Bard1* phenotypes, we performed deep RNA sequencing at
260 day 3 and day 6 of reprogramming. We called differentially expressed genes and found 753, 1555,
261 and 205 genes deregulated in respectively *Wdr5*, *Brca1* and *Bard1* knockdown cells following 3
262 days of OSKM induction (Fig. 6A). *Wdr5*, *Brca1* and *Bard1*-depleted cells showed reduced
263 expression of early pluripotency genes such as *Sall4*, *Cdh1* and *Epcam* (Fig. 6A).

264

265 Differentially expressed genes in each knockdown were further probed for overrepresented gene
266 ontology (GO) classes (Fig. 6B, Table S5). *Brca1* knockdown causes a reduction in gene expression
267 related to the cell cycle, response to DNA damage, and DNA repair (Fig. 6B). We asked whether the
268 effects on the DNA damage response (Fig. 5) are reflected in the transcriptome of *Wdr5* as well. To
269 test this, DNA repair genes were probed in a Gene Set Enrichment Analysis (GSEA) (Mootha et al.,
270 2003; Subramanian et al., 2005) comparing si*Wdr5* and control transcriptomes. Indeed, the
271 negative normalized enrichment score (NES) indicated decreased expression of DNA repair genes

272 in the siWdr5 as compared to the control (Fig. 6C, left). Furthermore, decreased expression of DNA
273 repair genes in siWdr5 was similar to that of siBrca1 and siBard1 (Fig. 6C, right and Fig.S6)

274
275 Wdr5 and Brca1 knockdowns shared a number of up regulated terms, including cell adhesion and
276 developmental processes (e.g. skeleton or blood vessel development) (Fig. 6B). Regulation of cell
277 proliferation is changed in Brca1, Bard1 and Wdr5 knockdowns; this GO term is enriched due to
278 increased expression of *Tgf β* , *Wnt*, *Bmp*, *Fgf* growth factors (Table S6, Fig. 6D). These growth
279 factors decrease cell proliferation (Vega et al., 2004), but are also involved in epithelial to
280 mesenchymal transitions (EMT) (Barrallo-Gimeno and Nieto, 2005), potentially counteracting the
281 MET required for reprogramming. Several cell proliferation markers, such as *Pcna*, *Ki-67* and *Mcm2*
282 were decreased in all three knockdowns, while *p21 (Cdkn1a)* was up regulated (Fig. 6D). We
283 assessed the gene expression levels of mesenchymal and epithelial markers in the three
284 knockdowns and observed a clear increase in mesenchymal gene expression in the Wdr5, Brca1
285 and Bard1 knockdown cells relative to control cells (Fig. S6, Fig. 6E). Some epithelial genes were
286 decreased (*Cdh1*, *Epcam* and *Krt8*), whereas others did not change substantially or were increased
287 (Fig. 6E, Fig. S6).

288 Together, these data indicate that Wdr5, Brca1 and Bard1 not only cooperate in pluripotent colony
289 formation (Fig. 4), but also share a functional interaction in the MET and the expression of DNA
290 damage response genes during early reprogramming.

291

292

293 **DISCUSSION**

294

295 This study reports on the colony morphology phenotypes of 300 chromatin-associated factors and
296 on the transcriptome phenotypes of 30 factors during early reprogramming of fibroblasts towards
297 induced pluripotency, constituting a highly relevant resource. Moreover, we have characterized the
298 phenotypes involved in the mesenchymal-epithelial transition and the DNA damage response in
299 more detail, and find cooperative contributions of three genes, Wdr5, Brca1 and Bard1 in both
300 these processes.

301

302 Several complexes associated with the DNA damage response and replication are highly induced
303 early in reprogramming (Hansson et al., 2012) and the p53-pathway is activated in cells
304 harbouring substantial DNA damage (Marion et al., 2009). DNA damage may be associated with
305 senescence, which can be rapidly induced by oxidative stress (Ben-Porath and Weinberg, 2005;
306 d'Adda di Fagagna et al., 2003). It has been proposed that in vitro cell culture generates oxidative
307 stress (Halliwell, 2003) and this could lead to an accelerated senescence (Parrinello et al., 2003). In
308 agreement with this, low oxidizing conditions alleviate the reprogramming barrier imposed by
309 senescence (Utikal et al., 2009). Some aging hallmarks, such as eroded telomeres (Lapasset et al.,
310 2011; Marion and Blasco, 2010) and senescence-associated epigenetic marks (Ocampo et al.,
311 2016) are reset by OSKM reprogramming. Brca1-Bard1 and Wdr5 may therefore alleviate a
312 senescence-related block of reprogramming. In addition, the requirement of a DNA damage
313 response could be related to the faster proliferation rates acquired early on in reprogramming
314 (Polo et al., 2012; Ruiz et al., 2011). Embryonic stem cells, which proliferate in a similar fashion,
315 require additional genome surveillance mechanisms to cope with fast DNA replication (Ahuja et al.,
316 2016). The reduction in γ H2A.X that we observe during normal reprogramming, however, is not
317 common to all reprogramming systems (Gonzalez et al., 2013) and the observed differences could

318 be due to presence of vitamin C in our medium, as the addition of antioxidants reduces genomic
319 instability in reprogramming cultures (Ji et al., 2014).

320
321 We found a functional interaction of Wdr5, Brca1 and Bard1 in reprogramming. *Brca1*, *Bard1* could
322 be direct or indirect targets of the SET/MLL complexes, of which Wdr5 is a subunit. In line with this
323 possibility, ChIP analysis showed that Wdr5 binds regulatory regions of *Brca1*, *Bard1* and other
324 genes involved in repair (Ang et al., 2011). Moreover, *Brca1* and *Bard1* transcripts are down
325 regulated after silencing *Wdr5* (Fig. 4). In addition, not only are Brca1 and Bard1 involved in
326 mitotic spindle organization and checkpoint gene regulation (Jin et al., 2009; Joukov et al., 2006;
327 Wang et al., 2004), MLL/Wdr5 has been implicated in cell cycle regulation, mitotic progression and
328 proper chromosome segregation (Ali et al., 2014; Liu et al., 2010; Ali et al., 2017).

329
330 Our study adds to the notion that colony morphology is linked to pluripotency (Abagnale et al.,
331 2017; Kato et al., 2016; Narva et al., 2017) and is regulated by adhesion molecules, extracellular
332 matrix and cytoskeleton forces. Upon differentiation, these processes orchestrate morphological
333 changes such as loss of colony compaction, increase of cell area, colony flattening, together with
334 changes in the pluripotency network (Narva et al., 2017). Therefore, colony morphology is a very
335 important readout for reprogramming quality. Moreover, medium-high throughput screening of
336 such multi-dimensional phenotypes is very powerful to identify functional interactions between
337 genes. Brca1, Bard1 and Wdr5 depleted cells gave rise to fewer yet bigger, flat, symmetric colonies,
338 due to a failure to properly down regulate mesenchymal cell adhesion molecules (Fig. 6). In
339 addition, these cells fail to activate epithelial and early pluripotency genes. Our study links the DNA
340 damage response to the MET program early in reprogramming through Brca1-Bard1 and Wdr5.
341 Interestingly, the converse process of EMT may relate to DNA damage in kidney disease (Slaats et
342 al., 2014) and cancer cells in culture (Chiba et al., 2012). Future work will further explore these
343 relationships as well as gene-gene interactions that modify the phenotypical plasticity of
344 reprogramming to induced pluripotency.

345

346 **EXPERIMENTAL PROCEDURES**

347

348 **Data and Software Availability**

349 Sequencing data are available at the GEO repository Superseries number GSE118680.

350 The code to reproduce reprogramming facilitator predictions by machine learning is available at
351 <https://github.com/simonvh/facilitators-penalosa-ruiz/>. The code to reproduce the timeline
352 projection is available at <https://github.com/TimEveeustra/Time-Curve-Projection/> (doi:
353 10.5281/zenodo.1405746).

354

355 **MEF Reprogramming and culture media**

356 Passage 1-2 MEFs (mouse embryonic fibroblasts) were seeded at a density of 10,000 cells per cm².
357 Next day, MEFs were transduced at an MOI of 1 with Tet-STEMCCA lentivirus (Sommer et al.,
358 2009), rtTA (Addgene#20342) and 8 µg·mL⁻¹ polybrene. Next day (day 0), cells were transferred to
359 either 1% gelatin-coated plates or mitotically inactive feeder cells, in reprogramming medium
360 (Vidal et al., 2014).

361

362 **siRNA transfections and siRNA screenings**

363 A custom Silencer siRNA library targeting around 300 mouse genes encoding chromatin factors
364 was designed (Thermo Scientific/Ambion, Table S1) and distributed in 6 plates. Each gene in the
365 library was targeted with three different siRNAs, which were pooled for transfection. For the high-
366 content screening, the six pooled plates were transfected in quadruplicate. Every plate contained
367 the following controls: siOct4 (siPou5f1), siMyc, siTrp53 and seven non-targeting (nt) controls.
368 Reverse transfections in a 96-well plate format were performed as follows: 20 μL of transfection
369 mix was prepared in each well before adding the cell suspension. This transfection mix consisted of
370 40 nM of pooled siRNAs, and 0.26 μL RNAiMAX lipofectamine (Thermo Scientific) diluted in
371 Optimem (Thermo Scientific). After incubation for 10 minutes, 100 μL of cell suspension (3000-
372 6000 cells) were added to each well. For transfections in a 6-well plate format, the protocol was
373 scaled up accordingly. Before adding 1.8 mL cell suspension with 100,000 cells, 220 μL transfection
374 mix was incubated in the wells for 10 minutes. The transfection mix consisted of 4 μL RNAiMAX
375 and a final concentration of 40 nM siRNA, all diluted in Optimem.
376

377 **Immunostaining**

378 Cells were cultured in 96-well Cell Carrier plates for microscopy (Perkin Elmer). After 6 days of
379 reprogramming, cells were washed with PBS and fixed with 4% PFA for 15 min. After blocking and
380 permeabilization, samples were incubated overnight with mouse anti-Cdh1 (Cell Signaling, 14472)
381 and then with goat anti-mouse Alexa-488 for 2 hours. Staining with rabbit anti-Sall4 (Abcam,
382 ab29112) was done overnight, followed by 3 hours incubation with goat anti-rabbit Alexa 568 and
383 40 $\mu\text{g}\cdot\text{mL}^{-1}$ DAPI. After antibody incubations, the cells were washed twice with PBS.
384

385 **High-content image acquisition and feature selection**

386 Plates were imaged with an Opera High-content Screening System (Perkin Elmer) with a 4X air
387 lens. Images were imported into the Columbus software platform (PerkinElmer). To segment
388 colonies imaged on multiple z-planes, we used the maximum projection of z-planes. Sall4 staining
389 was used to find and segment the colonies. Automated image analysis was used for image region
390 segmentation and for extraction of shape and morphology features. Image regions touching the
391 edge were removed. For more details, see Supplementary Information. After extracting all features
392 for every plate from the automated pipeline, a Z-score normalization was applied per plate (Bakal
393 et al., 2007) based on the mean values per feature. To select relevant features, a feature-to-feature
394 Pearson correlation was calculated. Features with a high pairwise correlation (>0.8) were
395 considered redundant.
396

397 **RNA sequencing and analysis**

398 CEL-seq2 sample preparation (Hashimshony et al., 2016) was performed with a few adaptations
399 (see Supplemental Information). Transcripts were mapped to *Mus musculus* genome version mm10
400 with Bowtie2 (Langmead and Salzberg, 2012), UMI corrected using standard settings of the
401 CELseq2 pipeline (<https://github.com/yanailab/CEL-Seq-pipeline>), and matched to the
402 gencode.vM13.annotation transcriptome. To relate knockdown data points to the progression of
403 reprogramming, the transcriptomes were subjected to principal component analysis (PCA).
404 Principal components 1 and 2 (PC1, PC2) were swapped (x-axis: PC2) and all data (knockdown and
405 time series) were rotated 15 degrees. A second order polynomial curve was fitted to the time series
406 (day 2-7), and all data points were projected on this curve (script:
407 <https://doi.org/10.5281/zenodo.1405747>). For each data point, the projected x coordinate was

408 used as a proxy for time, whereas the distance to the fitted time line (calculated using Pythagoras'
409 theorem) was used as a proxy for gene expression differences unrelated to the process of
410 reprogramming. For normal RNA sequencing, Kapa-RNA HyperPrep kit with Ribo Erase was used
411 for ribosomal depletion and library preparation (Roche, Kapa Biosystems), starting with 200 ng of
412 total RNA. The libraries were amplified for 10 cycles, quantified with Qubit, checked for size
413 distribution (300 bp) by Bioanalyzer (Agilent), and subjected to qPCR analysis before and after
414 library preparation. Libraries were sequenced paired-end (Illumina NextSeq 500, read length 43
415 bp). Reads were aligned to the mouse genome (mm10) with STAR version 2.5.2b (Dobin et al.,
416 2013).

417

418 **FACS analysis of DNA damage**

419 Reprogramming MEFs were transfected with siRNAs in 6-well plates. After 3 days, cells were fixed
420 on ice with 1 % PFA for 15 minutes and incubated with 70 % ice-cold ethanol at -20 °C for two
421 hours. Samples were then incubated with 100 µL mouse anti-phospho-H2AX (Millipore, diluted
422 1:100 in 0.25 % BSA 0.3 % triton/PBS) overnight at 4 °C. Then, cells were washed and stained with
423 100 µL Alexa 488 Goat anti-rabbit 488 (diluted 1:500) for 2 hours at room temperature. Finally,
424 samples were incubated with propidium iodide (PI) overnight in the fridge and were sorted using
425 an FC 500 (Beckman Coulter) machine. Data analysis was done with Flowing software v.2.5. As
426 positive control, reprogramming MEFs were treated with 400 µg·mL⁻¹ mitomycin C for 3 days.

427

428 **Double knockdowns and functional interactions**

429 The observed Sall4-colony-ratio was calculated dividing the double-knockdown number of colonies
430 by the average number of colonies of the control (6 biological replicates). The expected Sall4-
431 colony ratio was calculated by multiplying the ratios of the single knockdowns (Mani et al., 2008).
432 A P-value < 0.05 (two-tailed T-test) was considered significant. See supplementary information for
433 details.

434

435 **AUTHOR CONTRIBUTIONS**

436 Conceptualization GJCV, KWM, GPR; Methodology GPR, VB, GJCV, KWM, CB, JCRS; Experiments GPR,
437 VB, JPG, SW, JVvV; Analysis GPR, VB, JPG, GJCV, SJvH, TEV; Writing the manuscript GPR, GJCV, KWM.

438

439 **ACKNOWLEDGEMENTS**

440 The authors thank Dei M. Elurbe for useful suggestions, help on data analysis and processing of the
441 sequencing files, Jessie A.G. van Buggenum for help with the colony counting script. Siebe van
442 Genesen, Katie Tremble and E. Janssen-Megens for valuable technical help. VB and CB are funded
443 by the Stand Up to Cancer campaign for Cancer Research UK, and Cancer Research UK Programme
444 Foundation Award to C.B. (C37275/1A20146).

445

446 **REFERENCES**

447

448 Abagnale, G., Sechi, A., Steger, M., Zhou, Q., Kuo, C.C., Aydin, G., Schalla, C., Muller-Newen, G., Zenke,
449 M., Costa, I.G., *et al.* (2017). Surface Topography Guides Morphology and Spatial Patterning of
450 Induced Pluripotent Stem Cell Colonies. *Stem cell reports* 9, 654-666.

- 451 Ahuja, A.K., Jodkowska, K., Teloni, F., Bizard, A.H., Zellweger, R., Herrador, R., Ortega, S., Hickson,
452 I.D., Altmeyer, M., Mendez, J., *et al.* (2016). A short G1 phase imposes constitutive replication stress
453 and fork remodelling in mouse embryonic stem cells. *Nature communications* 7, 10660.
- 454 Ali, A., Veeranki, S.N., Chinchole, A., and Tyagi, S. (2017). MLL/WDR5 Complex Regulates Kif2A
455 Localization to Ensure Chromosome Congression and Proper Spindle Assembly during Mitosis.
456 *Developmental cell* 41, 605-622 e607.
- 457 Ali, A., Veeranki, S.N., and Tyagi, S. (2014). A SET-domain-independent role of WRAD complex in
458 cell-cycle regulatory function of mixed lineage leukemia. *Nucleic acids research* 42, 7611-7624.
- 459 Ang, Y.S., Tsai, S.Y., Lee, D.F., Monk, J., Su, J., Ratnakumar, K., Ding, J., Ge, Y., Darr, H., Chang, B., *et al.*
460 (2011). Wdr5 mediates self-renewal and reprogramming via the embryonic stem cell core
461 transcriptional network. *Cell* 145, 183-197.
- 462 Bakal, C., Aach, J., Church, G., and Perrimon, N. (2007). Quantitative morphological signatures define
463 local signaling networks regulating cell morphology. *Science* 316, 1753-1756.
- 464 Barrallo-Gimeno, A., and Nieto, M.A. (2005). The Snail genes as inducers of cell movement and
465 survival: implications in development and cancer. *Development* 132, 3151-3161.
- 466 Ben-Porath, I., and Weinberg, R.A. (2005). The signals and pathways activating cellular senescence.
467 *Int J Biochem Cell Biol* 37, 961-976.
- 468 Boutros, M., Heigwer, F., and Laufer, C. (2015). Microscopy-Based High-Content Screening. *Cell* 163,
469 1314-1325.
- 470 Cacchiarelli, D., Trapnell, C., Ziller, M.J., Soumillon, M., Cesana, M., Karnik, R., Donaghey, J., Smith,
471 Z.D., Ratanasirintrao, S., Zhang, X., *et al.* (2015). Integrative Analyses of Human Reprogramming
472 Reveal Dynamic Nature of Induced Pluripotency. *Cell* 162, 412-424.
- 473 Chantzoura, E., Skylaki, S., Menendez, S., Kim, S.I., Johnsson, A., Linnarsson, S., Woltjen, K.,
474 Chambers, I., and Kaji, K. (2015). Reprogramming Roadblocks Are System Dependent. *Stem cell*
475 *reports* 5, 350-364.
- 476 Chen, J., Liu, H., Liu, J., Qi, J., Wei, B., Yang, J., Liang, H., Chen, Y., Chen, J., Wu, Y., *et al.* (2013). H3K9
477 methylation is a barrier during somatic cell reprogramming into iPSCs. *Nature genetics* 45, 34-42.
- 478 Chiba, N., Comaills, V., Shiotani, B., Takahashi, F., Shimada, T., Tajima, K., Winokur, D., Hayashida, T.,
479 Willers, H., Brachtel, E., *et al.* (2012). Homeobox B9 induces epithelial-to-mesenchymal transition-
480 associated radioresistance by accelerating DNA damage responses. *Proceedings of the National*
481 *Academy of Sciences of the United States of America* 109, 2760-2765.
- 482 d'Adda di Fagagna, F., Reaper, P.M., Clay-Farrace, L., Fiegler, H., Carr, P., Von Zglinicki, T., Saretzki,
483 G., Carter, N.P., and Jackson, S.P. (2003). A DNA damage checkpoint response in telomere-initiated
484 senescence. *Nature* 426, 194-198.

- 485 Dobin, A., Davis, C.A., Schlesinger, F., Drenkow, J., Zaleski, C., Jha, S., Batut, P., Chaisson, M., and
486 Gingeras, T.R. (2013). STAR: ultrafast universal RNA-seq aligner. *Bioinformatics* 29, 15-21.
- 487 Fischer, B., Sandmann, T., Horn, T., Billmann, M., Chaudhary, V., Huber, W., and Boutros, M. (2015).
488 A map of directional genetic interactions in a metazoan cell. *eLife* 4.
- 489 Gao, Y., Chen, J., Li, K., Wu, T., Huang, B., Liu, W., Kou, X., Zhang, Y., Huang, H., Jiang, Y., *et al.* (2013).
490 Replacement of Oct4 by Tet1 during iPSC induction reveals an important role of DNA methylation
491 and hydroxymethylation in reprogramming. *Cell stem cell* 12, 453-469.
- 492 Golipour, A., David, L., Liu, Y., Jayakumaran, G., Hirsch, C.L., Trcka, D., and Wrana, J.L. (2012). A late
493 transition in somatic cell reprogramming requires regulators distinct from the pluripotency
494 network. *Cell stem cell* 11, 769-782.
- 495 Gonzalez, F., Georgieva, D., Vanoli, F., Shi, Z.D., Stadtfeld, M., Ludwig, T., Jasin, M., and Huangfu, D.
496 (2013). Homologous recombination DNA repair genes play a critical role in reprogramming to a
497 pluripotent state. *Cell reports* 3, 651-660.
- 498 Halliwell, B. (2003). Oxidative stress in cell culture: an under-appreciated problem? *FEBS Lett* 540,
499 3-6.
- 500 Hansson, J., Rafiee, M.R., Reiland, S., Polo, J.M., Gehring, J., Okawa, S., Huber, W., Hochedlinger, K.,
501 and Krijgsveld, J. (2012). Highly coordinated proteome dynamics during reprogramming of somatic
502 cells to pluripotency. *Cell reports* 2, 1579-1592.
- 503 Hashimshony, T., Senderovich, N., Avital, G., Klochendler, A., de Leeuw, Y., Anavy, L., Gennert, D., Li,
504 S., Livak, K.J., Rozenblatt-Rosen, O., *et al.* (2016). CEL-Seq2: sensitive highly-multiplexed single-cell
505 RNA-Seq. *Genome biology* 17, 77.
- 506 Ji, J., Sharma, V., Qi, S., Guarch, M.E., Zhao, P., Luo, Z., Fan, W., Wang, Y., Mbabaali, F., Neculai, D., *et al.*
507 (2014). Antioxidant supplementation reduces genomic aberrations in human induced pluripotent
508 stem cells. *Stem cell reports* 2, 44-51.
- 509 Jin, S., Gao, H., Mazzacurati, L., Wang, Y., Fan, W., Chen, Q., Yu, W., Wang, M., Zhu, X., Zhang, C., *et al.*
510 (2009). BRCA1 interaction of centrosomal protein Nlp is required for successful mitotic
511 progression. *The Journal of biological chemistry* 284, 22970-22977.
- 512 Joukov, V., Groen, A.C., Prokhorova, T., Gerson, R., White, E., Rodriguez, A., Walter, J.C., and
513 Livingston, D.M. (2006). The BRCA1/BARD1 heterodimer modulates ran-dependent mitotic spindle
514 assembly. *Cell* 127, 539-552.
- 515 Kato, R., Matsumoto, M., Sasaki, H., Joto, R., Okada, M., Ikeda, Y., Kanie, K., Suga, M., Kinehara, M.,
516 Yanagihara, K., *et al.* (2016). Parametric analysis of colony morphology of non-labelled live human
517 pluripotent stem cells for cell quality control. *Scientific reports* 6, 34009.
- 518 Langmead, B., and Salzberg, S.L. (2012). Fast gapped-read alignment with Bowtie 2. *Nature*
519 *methods* 9, 357-359.

- 520 Lapasset, L., Milhavet, O., Prieur, A., Besnard, E., Babled, A., Ait-Hamou, N., Leschik, J., Pellestor, F.,
521 Ramirez, J.M., De Vos, J., *et al.* (2011). Rejuvenating senescent and centenarian human cells by
522 reprogramming through the pluripotent state. *Genes & development* 25, 2248-2253.
- 523 Li, R., Liang, J., Ni, S., Zhou, T., Qing, X., Li, H., He, W., Chen, J., Li, F., Zhuang, Q., *et al.* (2010). A
524 mesenchymal-to-epithelial transition initiates and is required for the nuclear reprogramming of
525 mouse fibroblasts. *Cell stem cell* 7, 51-63.
- 526 Liu, H., Takeda, S., Kumar, R., Westergard, T.D., Brown, E.J., Pandita, T.K., Cheng, E.H., and Hsieh, J.J.
527 (2010). Phosphorylation of MLL by ATR is required for execution of mammalian S-phase
528 checkpoint. *Nature* 467, 343-346.
- 529 Maherali, N., Sridharan, R., Xie, W., Utikal, J., Eminli, S., Arnold, K., Stadtfeld, M., Yachechko, R.,
530 Tchieu, J., Jaenisch, R., *et al.* (2007). Directly reprogrammed fibroblasts show global epigenetic
531 remodeling and widespread tissue contribution. *Cell stem cell* 1, 55-70.
- 532 Mani, R., St Onge, R.P., Hartman, J.L.t., Giaever, G., and Roth, F.P. (2008). Defining genetic
533 interaction. *Proceedings of the National Academy of Sciences of the United States of America* 105,
534 3461-3466.
- 535 Marion, R.M., and Blasco, M.A. (2010). Telomere rejuvenation during nuclear reprogramming.
536 *Current opinion in genetics & development* 20, 190-196.
- 537 Marion, R.M., Strati, K., Li, H., Murga, M., Blanco, R., Ortega, S., Fernandez-Capetillo, O., Serrano, M.,
538 and Blasco, M.A. (2009). A p53-mediated DNA damage response limits reprogramming to ensure
539 iPS cell genomic integrity. *Nature* 460, 1149-1153.
- 540 Meissner, A., Mikkelsen, T.S., Gu, H., Wernig, M., Hanna, J., Sivachenko, A., Zhang, X., Bernstein, B.E.,
541 Nusbaum, C., Jaffe, D.B., *et al.* (2008). Genome-scale DNA methylation maps of pluripotent and
542 differentiated cells. *Nature* 454, 766-770.
- 543 Mikkelsen, T.S., Ku, M., Jaffe, D.B., Issac, B., Lieberman, E., Giannoukos, G., Alvarez, P., Brockman, W.,
544 Kim, T.K., Koche, R.P., *et al.* (2007). Genome-wide maps of chromatin state in pluripotent and
545 lineage-committed cells. *Nature* 448, 553-560.
- 546 Mulder, K.W., Wang, X., Escriu, C., Ito, Y., Schwarz, R.F., Gillis, J., Sirokmany, G., Donati, G., Uribe-
547 Lewis, S., Pavlidis, P., *et al.* (2012). Diverse epigenetic strategies interact to control epidermal
548 differentiation. *Nature cell biology* 14, 753-763.
- 549 Narva, E., Stubb, A., Guzman, C., Blomqvist, M., Balboa, D., Lerche, M., Saari, M., Otonkoski, T., and
550 Ivaska, J. (2017). A Strong Contractile Actin Fence and Large Adhesions Direct Human Pluripotent
551 Colony Morphology and Adhesion. *Stem cell reports* 9, 67-76.
- 552 Ocampo, A., Reddy, P., Martinez-Redondo, P., Platero-Luengo, A., Hatanaka, F., Hishida, T., Li, M.,
553 Lam, D., Kurita, M., Beyret, E., *et al.* (2016). In Vivo Amelioration of Age-Associated Hallmarks by
554 Partial Reprogramming. *Cell* 167, 1719-1733 e1712.

- 555 Onder, T.T., Kara, N., Cherry, A., Sinha, A.U., Zhu, N., Bernt, K.M., Cahan, P., Marcarci, B.O.,
556 Unternaehrer, J., Gupta, P.B., *et al.* (2012). Chromatin-modifying enzymes as modulators of
557 reprogramming. *Nature* *483*, 598-602.
- 558 Panopoulos, A.D., Yanes, O., Ruiz, S., Kida, Y.S., Diep, D., Tautenhahn, R., Herrerias, A., Batchelder,
559 E.M., Plongthongkum, N., Lutz, M., *et al.* (2012). The metabolome of induced pluripotent stem cells
560 reveals metabolic changes occurring in somatic cell reprogramming. *Cell research* *22*, 168-177.
- 561 Parrinello, S., Samper, E., Krtolica, A., Goldstein, J., Melov, S., and Campisi, J. (2003). Oxygen
562 sensitivity severely limits the replicative lifespan of murine fibroblasts. *Nature cell biology* *5*, 741-
563 747.
- 564 Polo, J.M., Anderssen, E., Walsh, R.M., Schwarz, B.A., Nefzger, C.M., Lim, S.M., Borkent, M., Apostolou,
565 E., Alaei, S., Cloutier, J., *et al.* (2012). A molecular roadmap of reprogramming somatic cells into iPSC
566 cells. *Cell* *151*, 1617-1632.
- 567 Qin, H., Diaz, A., Blouin, L., Lebbink, R.J., Patena, W., Tanbun, P., LeProust, E.M., McManus, M.T., Song,
568 J.S., and Ramalho-Santos, M. (2014). Systematic identification of barriers to human iPSC generation.
569 *Cell* *158*, 449-461.
- 570 Ruiz, S., Panopoulos, A.D., Herrerias, A., Bissig, K.D., Lutz, M., Berggren, W.T., Verma, I.M., and
571 Izpisua Belmonte, J.C. (2011). A high proliferation rate is required for cell reprogramming and
572 maintenance of human embryonic stem cell identity. *Current biology : CB* *21*, 45-52.
- 573 Samavarchi-Tehrani, P., Golipour, A., David, L., Sung, H.K., Beyer, T.A., Datti, A., Woltjen, K., Nagy, A.,
574 and Wrana, J.L. (2010). Functional genomics reveals a BMP-driven mesenchymal-to-epithelial
575 transition in the initiation of somatic cell reprogramming. *Cell stem cell* *7*, 64-77.
- 576 Sero, J.E., and Bakal, C. (2017). Multiparametric Analysis of Cell Shape Demonstrates that beta-PIX
577 Directly Couples YAP Activation to Extracellular Matrix Adhesion. *Cell systems* *4*, 84-96 e86.
- 578 Sharma, A., Singh, K., and Almasan, A. (2012). Histone H2AX phosphorylation: a marker for DNA
579 damage. *Methods in molecular biology* *920*, 613-626.
- 580 Silva, J., Barrandon, O., Nichols, J., Kawaguchi, J., Theunissen, T.W., and Smith, A. (2008). Promotion
581 of reprogramming to ground state pluripotency by signal inhibition. *PLoS biology* *6*, e253.
- 582 Slaats, G.G., Ghosh, A.K., Falke, L.L., Le Corre, S., Shaltiel, I.A., van de Hoek, G., Klasson, T.D., Stokman,
583 M.F., Logister, I., Verhaar, M.C., *et al.* (2014). Nephronophthisis-associated CEP164 regulates cell
584 cycle progression, apoptosis and epithelial-to-mesenchymal transition. *PLoS genetics* *10*,
585 e1004594.
- 586 Sommer, C.A., Stadtfeld, M., Murphy, G.J., Hochedlinger, K., Kotton, D.N., and Mostoslavsky, G.
587 (2009). Induced pluripotent stem cell generation using a single lentiviral stem cell cassette. *Stem*
588 *cells* *27*, 543-549.

- 589 Soufi, A., Donahue, G., and Zaret, K.S. (2012). Facilitators and impediments of the pluripotency
590 reprogramming factors' initial engagement with the genome. *Cell* *151*, 994-1004.
- 591 Sridharan, R., Gonzales-Cope, M., Chronis, C., Bonora, G., McKee, R., Huang, C., Patel, S., Lopez, D.,
592 Mishra, N., Pellegrini, M., *et al.* (2013). Proteomic and genomic approaches reveal critical functions
593 of H3K9 methylation and heterochromatin protein-1gamma in reprogramming to pluripotency.
594 *Nature cell biology* *15*, 872-882.
- 595 Takahashi, K., and Yamanaka, S. (2006). Induction of pluripotent stem cells from mouse embryonic
596 and adult fibroblast cultures by defined factors. *Cell* *126*, 663-676.
- 597 Utikal, J., Polo, J.M., Stadtfeld, M., Maherali, N., Kulalart, W., Walsh, R.M., Khalil, A., Rheinwald, J.G.,
598 and Hochedlinger, K. (2009). Immortalization eliminates a roadblock during cellular
599 reprogramming into iPS cells. *Nature* *460*, 1145-1148.
- 600 Vega, S., Morales, A.V., Ocana, O.H., Valdes, F., Fabregat, I., and Nieto, M.A. (2004). Snail blocks the
601 cell cycle and confers resistance to cell death. *Genes & development* *18*, 1131-1143.
- 602 Vidal, S.E., Amlani, B., Chen, T., Tsirigos, A., and Stadtfeld, M. (2014). Combinatorial modulation of
603 signaling pathways reveals cell-type-specific requirements for highly efficient and synchronous
604 iPSC reprogramming. *Stem cell reports* *3*, 574-584.
- 605 Wang, C., Lee, J.E., Lai, B., Macfarlan, T.S., Xu, S., Zhuang, L., Liu, C., Peng, W., and Ge, K. (2016).
606 Enhancer priming by H3K4 methyltransferase MLL4 controls cell fate transition. *Proceedings of the*
607 *National Academy of Sciences of the United States of America* *113*, 11871-11876.
- 608 Wang, R.H., Yu, H., and Deng, C.X. (2004). A requirement for breast-cancer-associated gene 1
609 (BRCA1) in the spindle checkpoint. *Proceedings of the National Academy of Sciences of the United*
610 *States of America* *101*, 17108-17113.
- 611 Wang, X., Castro, M.A., Mulder, K.W., and Markowetz, F. (2012). Posterior association networks and
612 functional modules inferred from rich phenotypes of gene perturbations. *PLoS computational*
613 *biology* *8*, e1002566.
- 614 Wu, L.C., Wang, Z.W., Tsan, J.T., Spillman, M.A., Phung, A., Xu, X.L., Yang, M.C., Hwang, L.Y., Bowcock,
615 A.M., and Baer, R. (1996). Identification of a RING protein that can interact in vivo with the BRCA1
616 gene product. *Nature genetics* *14*, 430-440.
- 617 Zhang, P., Wei, Y., Wang, L., Debeb, B.G., Yuan, Y., Zhang, J., Yuan, J., Wang, M., Chen, D., Sun, Y., *et al.*
618 (2014). ATM-mediated stabilization of ZEB1 promotes DNA damage response and radioresistance
619 through CHK1. *Nature cell biology* *16*, 864-875.
- 620 Zhuang, Q., Li, W., Benda, C., Huang, Z., Ahmed, T., Liu, P., Guo, X., Ibanez, D.P., Luo, Z., Zhang, M., *et*
621 *al.* (2018). NCoR/SMRT co-repressors cooperate with c-MYC to create an epigenetic barrier to
622 somatic cell reprogramming. *Nature cell biology* *20*, 400-412.
623
624

625 **FIGURE TITLES AND LEGENDS**

626

627 **Figure 1. High throughput analysis of the early phase of reprogramming**

628 (A) Experimental design of high content imaging siRNA screen. (B) Immunofluorescence of pre-iPS
629 colonies at day 6 stained for pluripotency markers Cdh1 and Sall4 with DAPI counterstain. Scale
630 bar represents 50 μm . (C) Comparison of colony phenotypes of control, siMyc and siOct4 cells at
631 reprogramming day 6, stained for Sall4 and Cdh1. The scale bar represents 100 μm (left) and the
632 images on the right are a 4x zoom-in from the inset squares on the left. (D) siRNAs in the whole
633 screen ranked from low to high z-scores, based on the number of colonies. Positive controls are
634 highlighted in colors. Each siRNA represents the average z-score from four replicates.

635

636 **Figure 2. High-content microscopy screen reveals five major phenotypes of colony**
637 **formation**

638 (A) An average Z-score for selected features was calculated from the quadruplicates and
639 represented in a heatmap. Features are clustered by Euclidean distance and rows are clustered by
640 K-means. The bar on the left represents the cluster number and the gene symbols on the right are
641 the hits of the screen and the controls. In brackets are the number of controls in a particular
642 cluster. (B) Example images of knockdowns depicting different phenotypes. Scale bar is 200 μm
643 (C). Pluripotency-associated hits were selected based on a combination of a probability prediction
644 by machine learning, based on known reprogramming facilitators, and a correlation analysis with
645 the positive and negative controls. Selected top-hits are colored according to the cluster number
646 (panel A, cf. Table S4, Fig. S2).

647

648 **Figure 3. Transcriptome-based secondary screening**

649 (A) Selected hits (30) and controls were transfected in triplicate and cultured until reprogramming
650 day 6. The transcriptomes were analyzed together with a time series of control cells. (B) siRNA-to-
651 siRNA Pearson correlation heatmaps based on transcriptomes. (C) Scatter plot representing
652 pairwise siRNA correlations of transcriptomes (x-axis) and high-content image analysis (y-axis).
653 siRNA pairs with highest correlations in both approaches are highlighted. (D) Analysis of the
654 progression of reprogramming in knockdown cells compared to cells of the time course, based on
655 PCA analysis of the transcriptomes and the projection of all data points on a curve fitted to the time
656 course. (E) Boxplots representing log transformed and normalized gene expression values from the
657 CELSeq2 time-course dataset. Each color depicts different groups of genes. (Experimental
658 procedures, cf. Fig. S3 and Fig. S4).

659

660 **Figure 4. Brca1, Bard1 and Wdr5 functionally interact in early reprogramming**

661 (A) Gene expression of Brca1, Bard1 and Wdr5 measured by RT-qPCR. Fold change was calculated
662 relative to MEFs (day 0) gene expression. Each data point represents the mean value \pm standard
663 deviation of a biological duplicate. (B) Dot plot representing the number of Sall4-positive colonies
664 measured by in-cell western in control and Brca1, Bard1 and Wdr5 knockdowns at day 6. Each dot
665 represents one biological replicate and statistical significance determined by ANOVA is
666 represented as *** $p < 0.0005$. (C) Sall-4 colony ratios of the single and double knockdowns
667 compared to the non-targeting (nt) control, measured by in-cell western. Functional interaction is
668 determined by comparing the mean difference in double knockdown colony ratios: observed vs.
669 expected. Each dot represents one biological replicate of an in-cell western for colonies at day 6

670 stained for Sall-4. Statistical significance $p < 0.05$ (*) was calculated with two tailed T-test. **(D)** Dot
671 plots to show Wdr5, Brca1 or Bard1 gene expression as counts per million reads (cpm) in siBard1,
672 siBrca1, siWdr5 and nt control.

673

674 **Figure 5. Wdr5, Bard1 and Brca1 are functionally connected in the DNA damage response**
675 **pathway**

676 **(A)** Representative FACS histograms showing the cell distribution with log-intensity of γ H2AX in
677 reprogramming populations measured in different conditions (white, nt; purple, siRNA). **(B)**
678 Dotplot representing the quantification of γ H2AX -positive cells in each condition in biological
679 replicates. Each of the data points corresponds to a biological replicate, measured from
680 independent experiments. Statistical significance was determined by one-way ANOVA. $p < 0.05$ (*),
681 $p < 0.005$ (**) and $p < 0.0005$ (***). **(C)** Confocal images of reprogramming cells at day 3, stained for
682 γ H2AX (green) SSEA1 (red), counterstained with DAPI. Scale bar is 100 μ m. See also Fig. S5.

683

684 **Figure 6. Wdr5, Brca1 and Bard1 depletion affects expression profiles of MET and DNA**
685 **repair genes**

686 **(A)** Volcano plots for siBard1 (left), siBrca1 (middle) and siWdr5 differential gene expression at
687 reprogramming day 3. Blue highlight: differentially expressed genes (\log_2 -fold change ≥ 1 , adjusted
688 p-value < 0.05). **(B)** Bubble plot, showing examples of some of the most enriched terms
689 (upregulated genes, orange; downregulated genes, blue) after Gene Ontology functional
690 classification in siWdr5 and siBrca1 at Day 3. Bubble sizes represent the number of genes. **(C)** Gene
691 Set Enrichment Analysis for DNA repair by homologous recombination (HR) comparing siWdr5 vs.
692 control transcriptomes (left). Heatmap for siBrca1, siBard1 and siWdr5 samples showing DNA
693 repair by HR genes, represented as \log_2 -ratio relative to control (right) **(D)** Dot plots for signaling
694 genes (magenta) and cell proliferation markers (yellow) quantified as counts per million reads
695 (cpm) in control, siBrca1, siBard1 and siWdr5 cells. **(E)** Heatmap representing the \log_2 -ratio of
696 mesenchymal and epithelial gene expression of the three knockdowns relative to control. See also
697 Fig. S6 and Table S5.

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FIGURE 1

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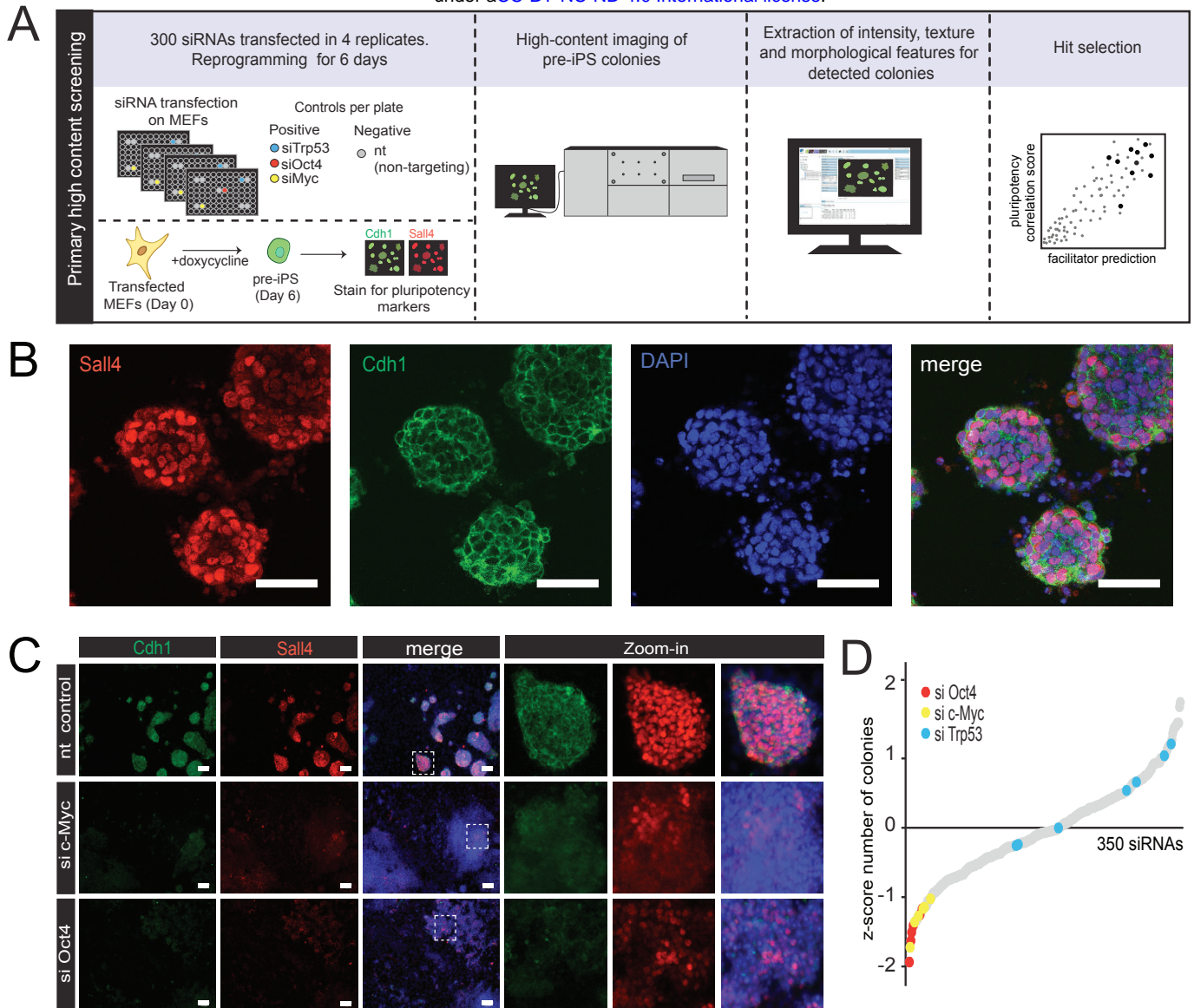


FIGURE 2

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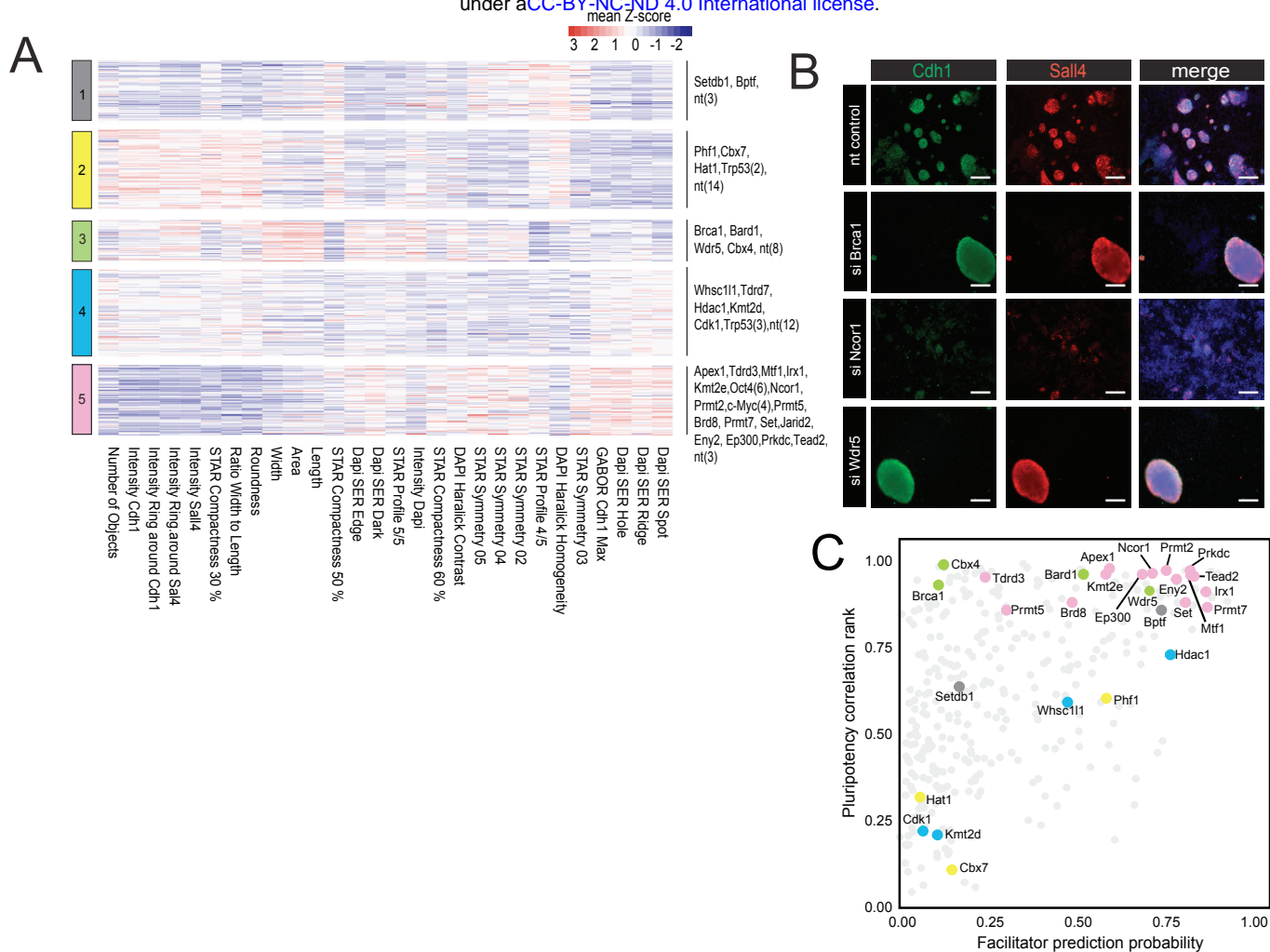


FIGURE 3

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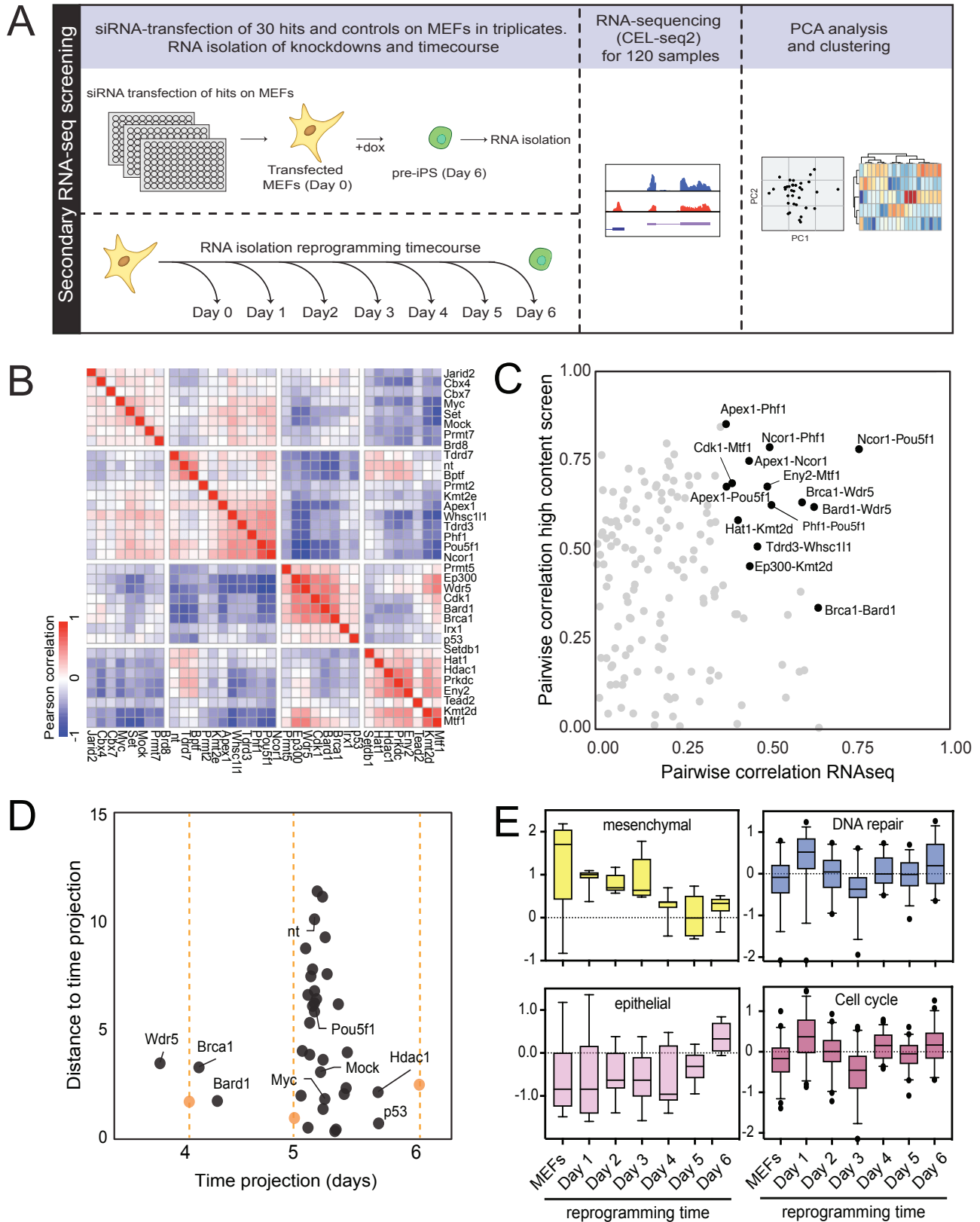


FIGURE 4

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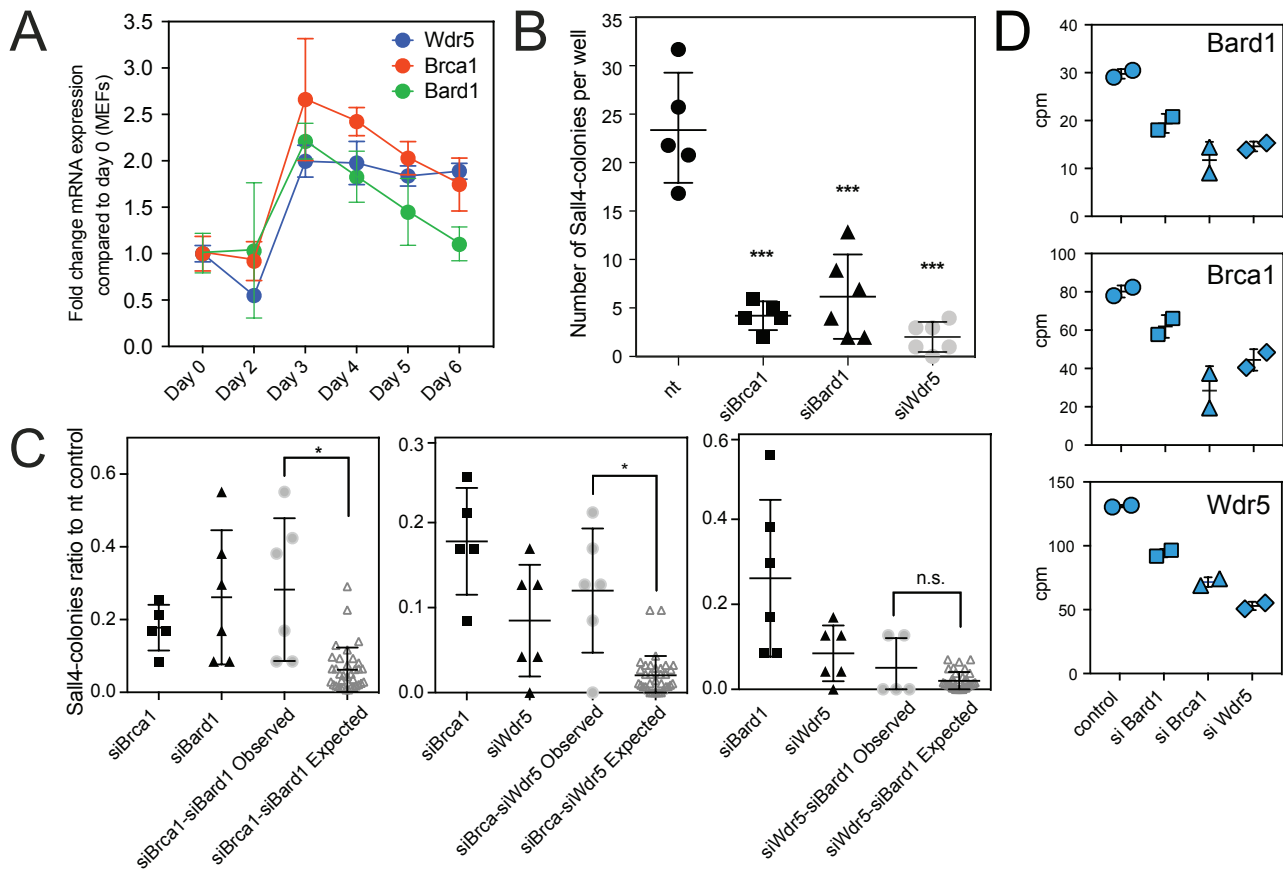


FIGURE 5

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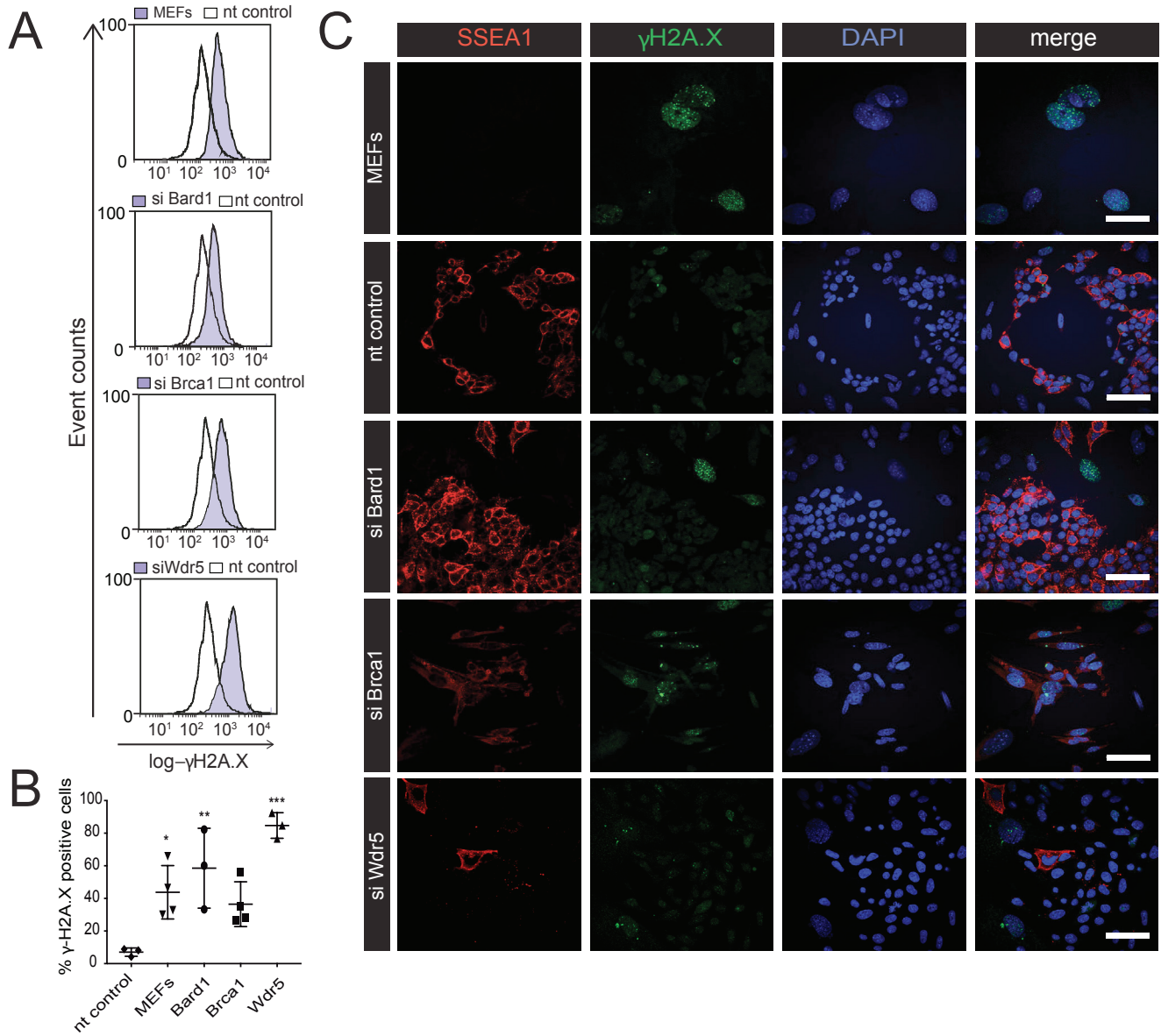


FIGURE 6

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