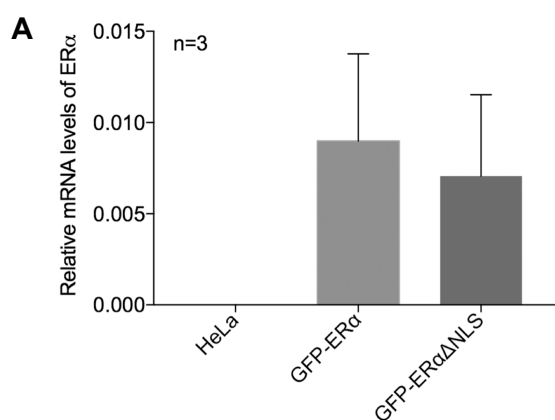


1 **Supplementary Material: Automated quantification**
 2 **of extranuclear ER α using phosphor-integrated dots**
 3 **for predicting endocrine therapy resistance in**
 4 **HR+/HER2- breast cancer**

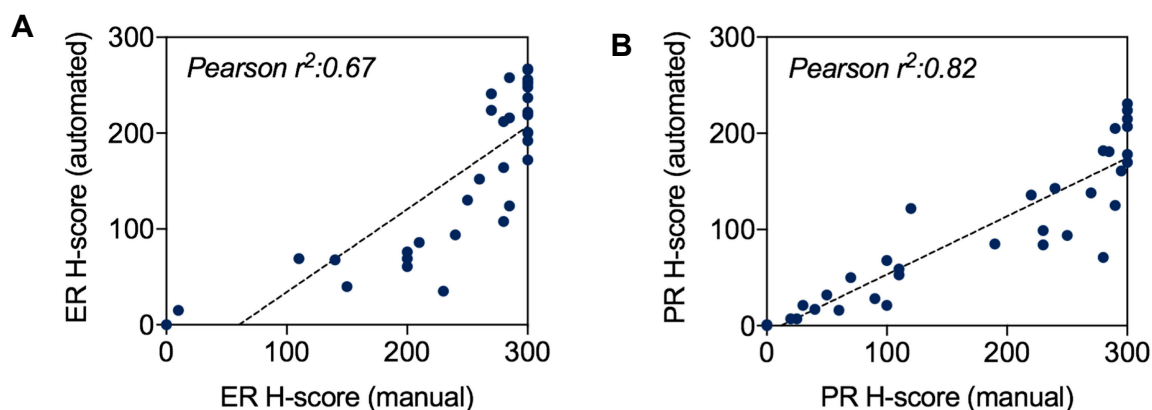
5 Zhaorong Guo, Hiroshi Tada, Narufumi Kitamura, Yoh Hamada, Minoru Miyashita, Narumi
 6 Harada, Akiko Sato, Yohei Hamanaka, Kouki Tsuboi, Nobuhisa Harada, Mayumi Takano-
 7 Kasuya, Hisatake Okada, Yasushi Nakano, Noriaki Ohuchi, Shin-ichi Hayashi, Takanori Ishida¹
 8 Kohsuke Gonda

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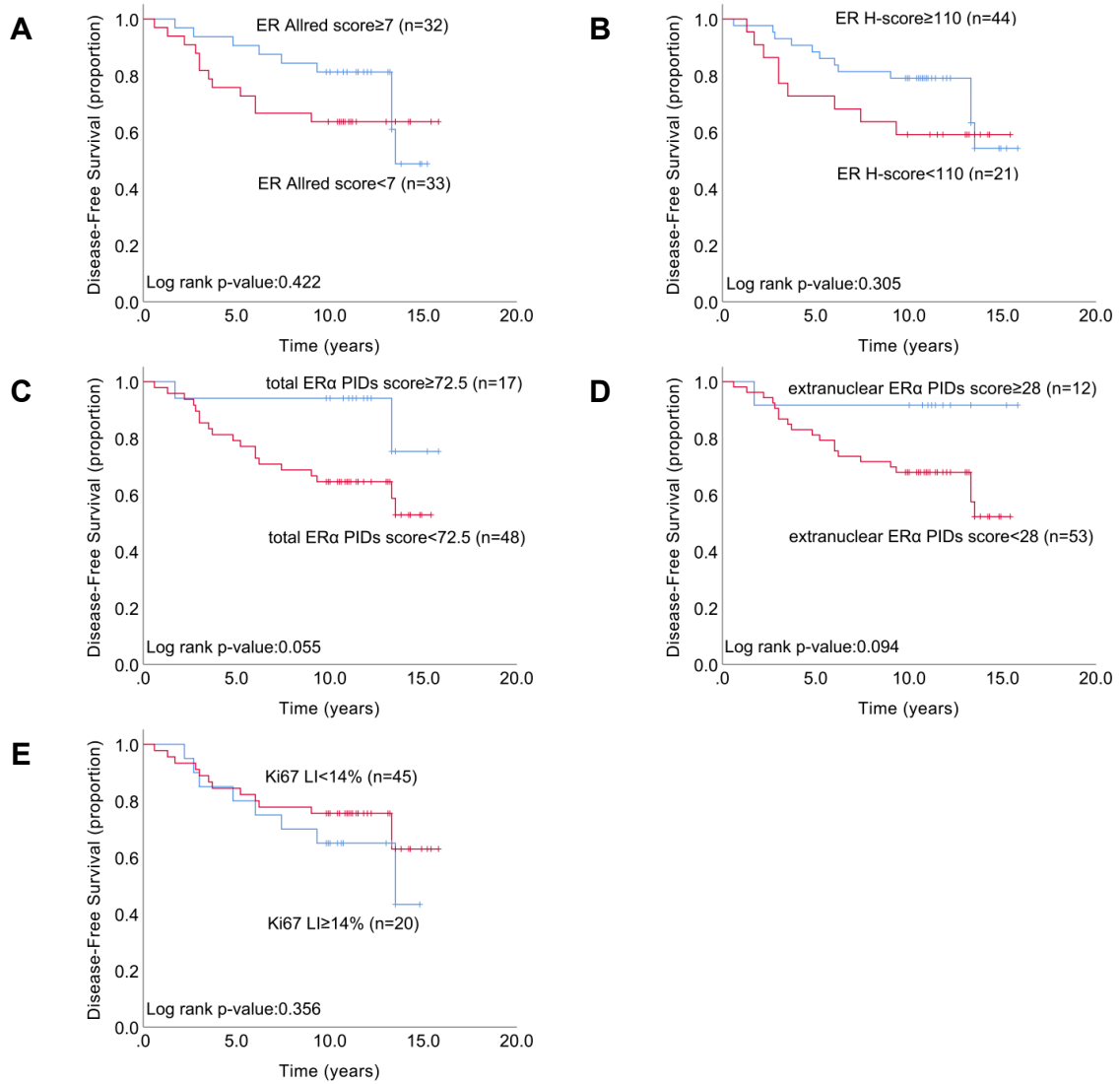
11 **Figure S1.** Verification of transfection with GFP-ER α or GFP-ER α ΔNLS expression plasmids. Relative
 12 copy number of ER α mRNA expression level for HeLa cells, GFP-ER α plasmids-transfected HeLa
 13 cells, GFP-ER α ΔNLS plasmids-transfected HeLa cells.



14

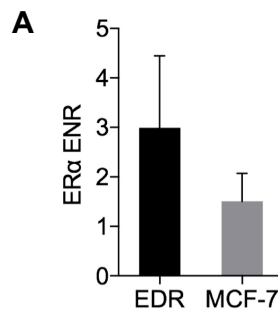
15 **Figure S2.** H-scores for the assessment of ER and PR are highly reproducible. The H-scores of
 16 randomly selected 34 cases were assessed with the Aperio image analysis system, which has a strong
 17 correlation with manual scoring evaluation by an expert pathologist. Pearson coefficients $r^2=0.67$ (ER,
 18 A) and 0.82 (PR, B).

19



20

21 **Figure S3.** Kaplan-Meier analysis of DFS according to ERα expression. Kaplan-Meier analysis of DFS
 22 according to the cut-off values of the ER Allred score(A), ER H-score (B), total ERα PID score (C),
 23 extranuclear ERα PID score (D), Ki67 LI (E). P values were determined with the log-rank test.



24

25 **Figure S4 A** A comparison of ENR between EDR (estrogen-deprivation-resistant) cell and MCF-7 cell
 26 ($p= 0.066$)

27

28

29

Supplementary Table S1. Associations between ER α ENR expression and clinicopathological

Characteristics	No. (%) of Patients		<i>P</i> value
	ER α ENR<0.5 (n=30)	ER α ENR \geq 0.5 (n=35)	
Menopausal status			
pre	7 (11)	21 (32)	0.673
post	11 (17)	26 (40)	
Lymph node status			
N0	12 (18)	26 (40)	0.406
N \geq 1	6 (9)	21 (32)	
Stage			
I	9 (14)	20 (31)	0.674
II	8 (12)	21 (32)	
III	1 (2)	6 (9)	
Histological grade			
1	6 (9)	14 (22)	0.961
2	9 (14)	25 (38)	
3	3 (5)	8 (12)	
Ki67 LI (%)			
<14	13 (20)	32 (49)	0.746
\geq 14	5 (8)	15 (23)	
ER H-score			
<110	2 (3)	20 (31)	0.017
\geq 110	16 (25)	27 (41)	
PR H-score			
<100	11 (17)	25 (38)	0.565
\geq 100	7 (11)	22 (34)	

30



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