1 # Supplementary Table

2 **Table S1. List of antibodies.**

3 CALR: calreticulin; CD: cluster of differentiation; HER2: human epidermal growth factor
4 receptor 2; MCL1: myeloid leukemia cell differentiation protein 1; N.A.: not applicable.

5

Target	**Host**	**Conjugate**	**Clone**	**Company**
Anti-mouse IgG	Goat	Alexa Fluor® 488	oligoclonal	Invitrogen
Anti-rabbit IgG	Goat	Alexa Fluor® 488	oligoclonal	Invitrogen
CALR	Mouse	unconjugated	FMC 75	Abcam
CD14	Mouse	APC	61D3	eBioscience
CD16	Mouse	PE	3G8	Beckman Coulter
CD32A	Rabbit	unconjugated	polyclonal	Abcam
CD32B	Rabbit	unconjugated	polyclonal	Abcam
CD47	Mouse	unconjugated	B6H12	eBioscience
CD64	Mouse	PC5	22	Beckman Coulter
HER2	Mouse	unconjugated	N.A.	Roche
MCL1	Rabbit	unconjugated	D35A5	Cell Signaling
Zombie Violet	N.A.	N.A.	N.A.	BioLegend

6

7 # Supplementary figures

8 **Fig. 1** Workflow diagram of the ADCP/ADCC assay**.** SKBR3 cells were stained with CFSE
9 and incubated with PBMCs at an E:T ratio of 25:1 and incubated for 2.5 h with or without
10 trastuzumab. Subsequently, cells were stained with a life/dead staining (Zombie Violet™),
11 following effector cell staining (anti-CD14). Staining events were quantified by FCM. CFSE+
12 Zombie Violet+ cells were classified as lysed SKBR3 cells by ADCC and CFSE+ CD14+
13 cells as phagocytosed SKBR3 cells by ADCP. CFSE: carboxyfluorescein diacetate

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1 succinimidyl ester, FCM: flow cytometry; h: hours; HDACi: histone deacetylase inhibitors;
2 E:T: effector to target; PBMCs: peripheral blood mononuclear cells
3
4 **Fig. S2** Titration of valproic acid and vorinostat. SKBR3 cells were treated with (**a**) VPA or (**b**)
5 SAHA, for 24 h. Subsequently, cells were stained with Annexin V and PI. Staining events
6 were quantified by FCM. Early apoptotic cells were determined as Annexin V positive and PI
7 negative (Annexin V+ / PI-). % Annexin V+ / PI- is depicted on the ordinate, different
8 treatments and corresponding concentrations are indicated on the abscissa. Mean %
9 Annexin V+ / PI- is illustrated by dots ± SEM by short horizontal lines connected with a
10 vertical line (3 individual experiments). FCM: flow cytometry; h: hours; PI: propidium iodide,
11 SAHA: vorinostat; SEM: standard error of the mean; VPA: valproic acid
12
13 **Fig. S3** Measurement of ADCC with flow cytometry (FCM) and imaging flow cytometry (IFC)
14 is correlated with each other. ADCP/ADCC assay was performed as described in Fig. S1 and
15 measured by FCM and IFC. (**a**) FCM gating strategy for ADCC. (**b**) IFC gating strategy
16 ADCC. (**c**) ADCC example images obtained by IFC. (**d**) Bland-Altman plots show the
17 difference (FCM-IFC), depicted on the ordinate, and the average ((FCM+IFC)/2),
18 represented at the abscissa, of the two different measurements. Dotted lines represent the
19 95% limits of agreement. Each dot represents one individual experiment. Bias (0.213) and
20 95% limits of agreement (-6.396 to 6.823). APC: allophycocyanin; CFSE: carboxyfluorescein
21 succinimidyl ester; FCM: flow cytometry; IFC: imaging flow cytometry; FS: forward scatter;
22 SS: side scatter
23
24 **Fig. S4** Valproic acid and vorinostat has no effect on trastuzumab-independent
25 phagocytosis. SKBR3 cells were pretreated with VPA or SAHA, for 24 h, following the
26 ADCP/ADCC assay as described in Fig. S1 and measured by FCM. (**a**, **c**, **e**) ADCP | Valproic
27 acid (**b**, **d**, **f**) ADCP | Vorinostat. (**c**-**f**) E:T ratio of 12:1. Results are illustrated by scatter plots.
28 Circles, boxes and triangles illustrate individual measured values. ADCP activity is depicted

1 on the ordinate, different treatments and corresponding concentrations are indicated on the
2 abscissa. Mean ADCP activity is illustrated by a long horizontal line ± SD by short horizontal
3 lines connected with a vertical line. SAHA: vorinostat; SD: standard deviation; Tras:
4 trastuzumab; VPA: valproic acid
5
6 **Fig. S5** Valproic acid and vorinostat has no effect on ADCC**.** SKBR3 cells were treated with
7 VPA or SAHA, for 24 h, following the ADCP/ADCC assay as described in Fig. S1 and
8 measured by FCM. (**a**, **c**, **e**) ADCC **|** Valproic acid (**b**, **d**, **f**) ADCC **|** Vorinostat. (**c**-**f**) E:T ratio
9 of 12:1. Results are illustrated by scatter plots. Circles, boxes and triangles illustrate
10 individual measured values. ADCC activity is depicted on the ordinate, different treatments
11 and corresponding concentrations are indicated on the abscissa. Mean ADCC activity is
12 illustrated by a long horizontal line ± SD by short horizontal lines connected with a vertical
13 line. SAHA: vorinostat; SD: standard deviation; VPA: valproic acid
14
15 **Fig. S6** Valproic acid and vorinostat decrease HER2 expression on tumor cells**.** SKBR3 cells
16 were treated with (**a**) VPA or (**b**) SAHA, for 24 h. Subsequently, cells were stained anti-HER2
17 (trastuzumab) following appropriate secondary antibody staining. Staining events were
18 quantified by FCM. Results are illustrated by scatter plots. Circles, boxes and triangles
19 illustrate individual measured values. Mean HER2 MFI is illustrated by a long horizontal line
20 ± SD by short horizontal lines connected with a vertical line. Corresponding overlay
21 histograms are illustrated. Differences were calculated by a RM one-way ANOVA and
22 Fisher's LSD post hoc test. **$p<0.01$; FI: fluorescence intensity; HER2: human epidermal
23 growth factor receptor 2; MFI: mean fluorescence intensity; VPA: valproic acid; SAHA:
24 vorinostat; SD: standard deviation
25
26 **Fig. S7** MCL1 knock-down has no effect on ADCC. (**a**) % ADCP after MCL1 knock-down. (**b**)
27 trastuzumab-independent cytotoxicity after MCL1 knock-down. (**c**) % ADCC after MCL1
28 knock-down in combination with trastuzumab. ADCP/ADCC assay was performed as

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described in Fig. S1 and measured by FCM. Results are illustrated by scatter plots. Circles, boxes and triangles illustrate individual measured values. ADCP and ADCC activity is depicted on the ordinate, different treatments and corresponding concentrations are indicated on the abscissa. Mean ADCP and ADCC activity is illustrated by a long horizontal line ± SD by short horizontal lines connected with a vertical line. Differences were calculated by a one-way ANOVA and Fisher's LSD post hoc test. **p<0.01; ***p<0.001; MCL1: myeloid leukemia cell differentiation protein 1; MFI: mean fluorescence intensity; SAHA: vorinostat; Scr: scrambled; siRNA: small interfering RNA; Tras: trastuzumab; VPA: valproic acid

Fig. S8 Valproic acid or vorinostat-induced secretome has no effect on ADCP or ADCC. SKBR3 cells were treated with VPA or SAHA, for 24 h. Afterwards PBMCs were stimulated with the VPA or SAHA-induced secretome (supernatant) of SKBR3 cells for 12 h, following the ADCP/ADCC assay as described in Fig. S1 and measured by FCM. (**a**, **c**) ADCP **|** Valproic acid (**b-d**) ADCP **|** Vorinostat (**e**, **g**) ADCC **|** Valproic acid (**f**, **h**) ADCC **|** Vorinostat. Results are illustrated by scatter plots. Circles, boxes and triangles illustrate individual measured values. % ADCP and ADCC is depicted on the ordinate, different treatments and corresponding concentrations are indicated on the abscissa. Mean % ADCP and ADCC is illustrated by a long horizontal line and ± SD by short horizontal lines connected with a vertical line. SAHA: vorinostat; SD: standard deviation; Tras: trastuzumab; VPA: valproic acid

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