**Supplementary information**

**Identification of small compounds regulating the secretion of extracellular vesicles via a TIM4-affinity ELISA**

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**Supplementary Figure S1.** AA2 inhibited EV secretion independent of caspase 3. (**a**–**c**) Jurkat cells were pre-treated with 0 or 50 μM Z-VAD(OMe)-FMK for 3 h, and then with 0 or 5 μM AA2 for 24 h. Cytotoxicity and cell growth were determined using lactate dehydrogenase (LDH) (**a**) and WST-8 (**b**) assays. (**c**) Secreted extracellular vesicles (EVs) were determined using a TIM4-CD81 ELISA. \*; *p* < 0.05, vs. DMSO, Student's *t*-test.

**Supplementary Figure S2.** Cucurbitacin B, gossypol, and obatoclax induced EV secretion in HEK293 cells. (**a**, **b**) HEK293 cells were treated with 1 μM cucurbitacin B, 0.3 μM gossypol, or 0.03 μM obatoclax for 24 h and then secreted EVs were determined using TIM4-CD9 and TIM4-CD63 ELISAs (**a**) or NTA (**b**). \*; *p* < 0.05, \*\*; *p* < 0.01, vs. DMSO, Student's *t*-test.