

## Supplementary Material

# Design and Development of a Fluorescent Imaging Agent for Vemurafenib to Probe Drug Activity in Melanoma

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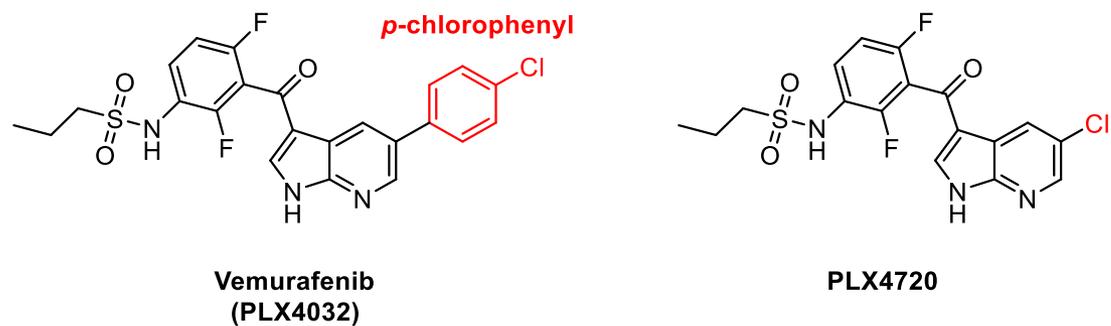
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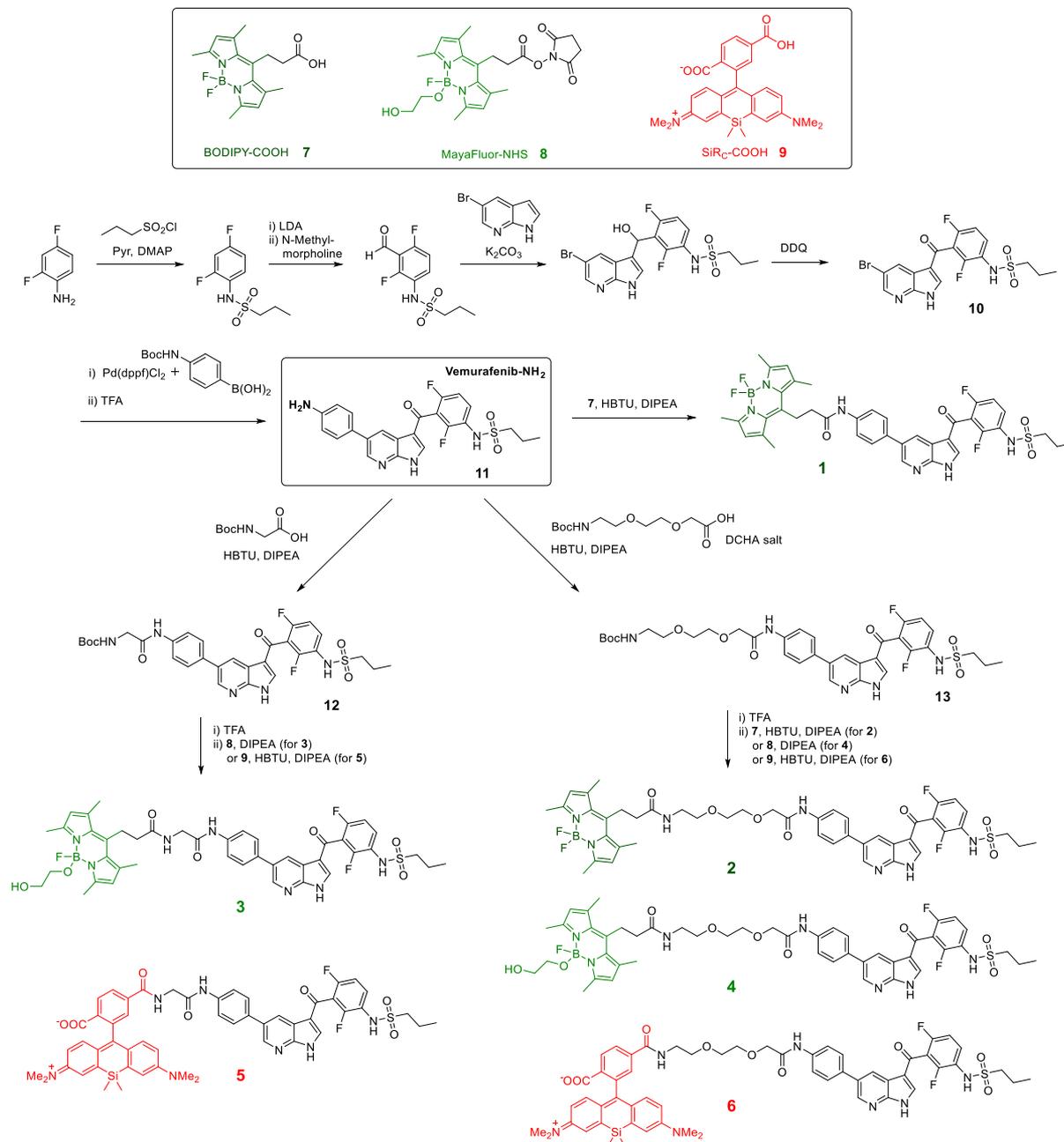
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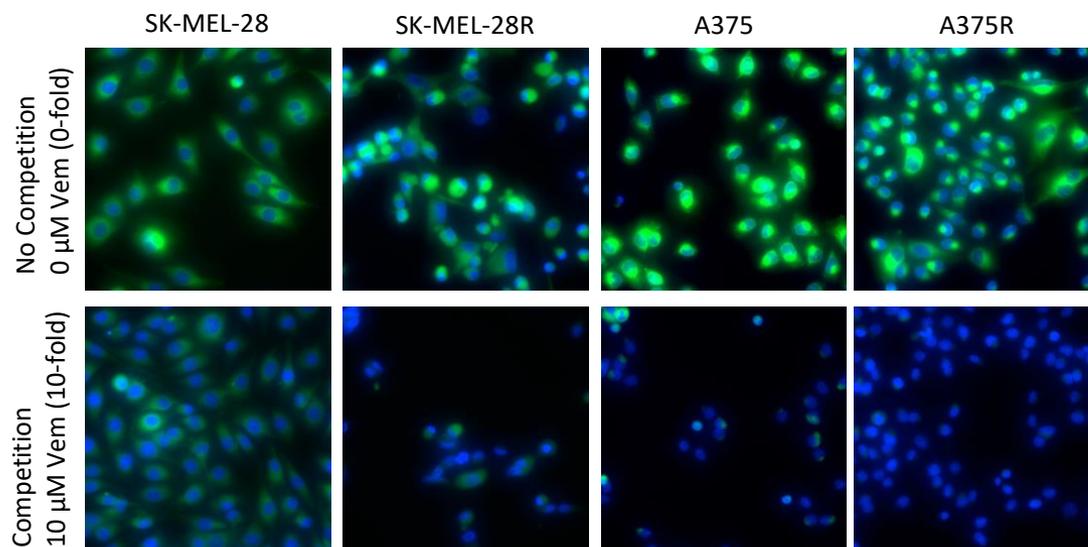
## Supporting Figures and Tables



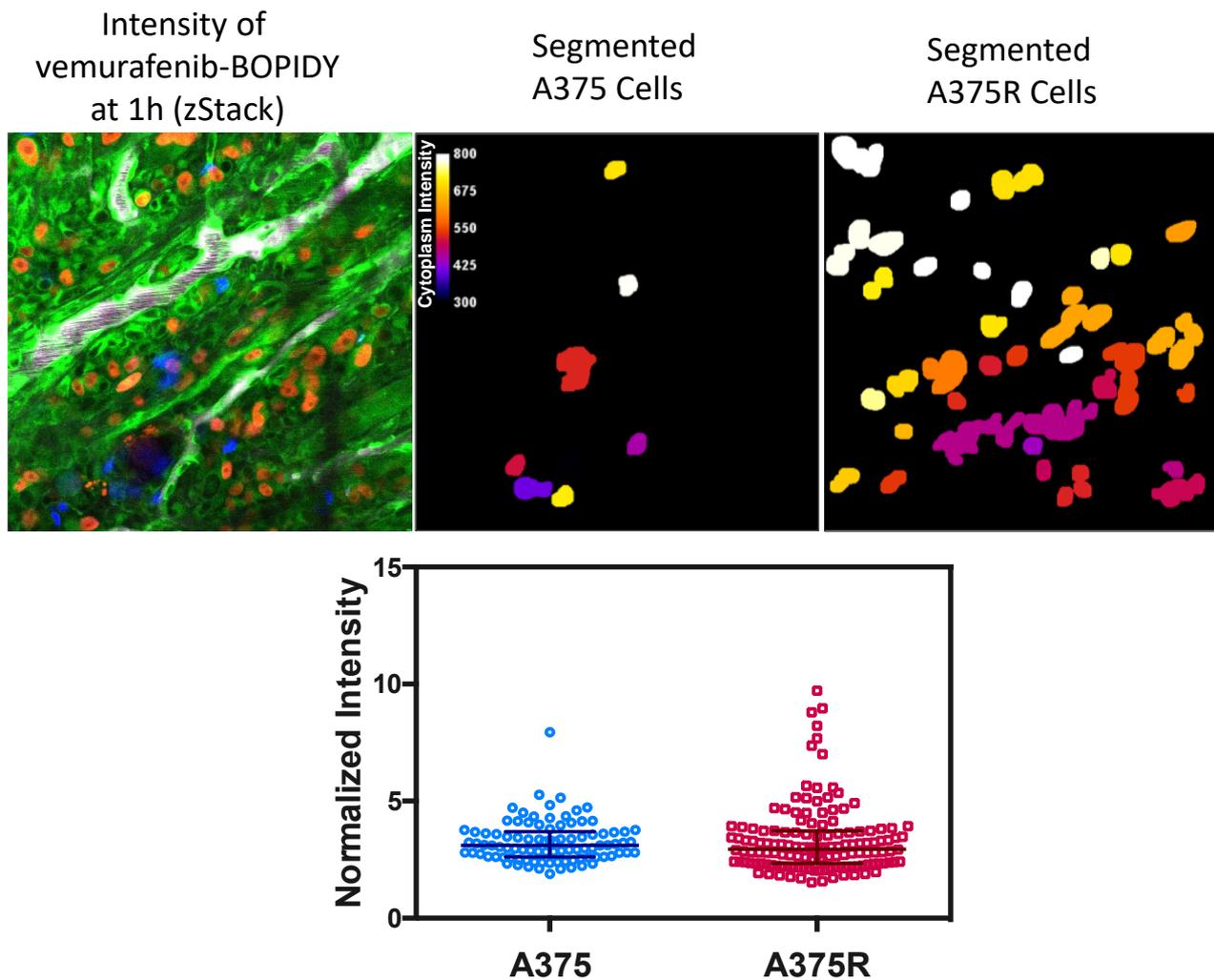
**Figure S1.** Chemical structures of vemurafenib and the similar BRAF inhibitor PLX4720 highlighting the *p*-chlorophenyl substituent, which provides increased hydrophobicity at the solvent site of the drug inside the binding pocket of the target.



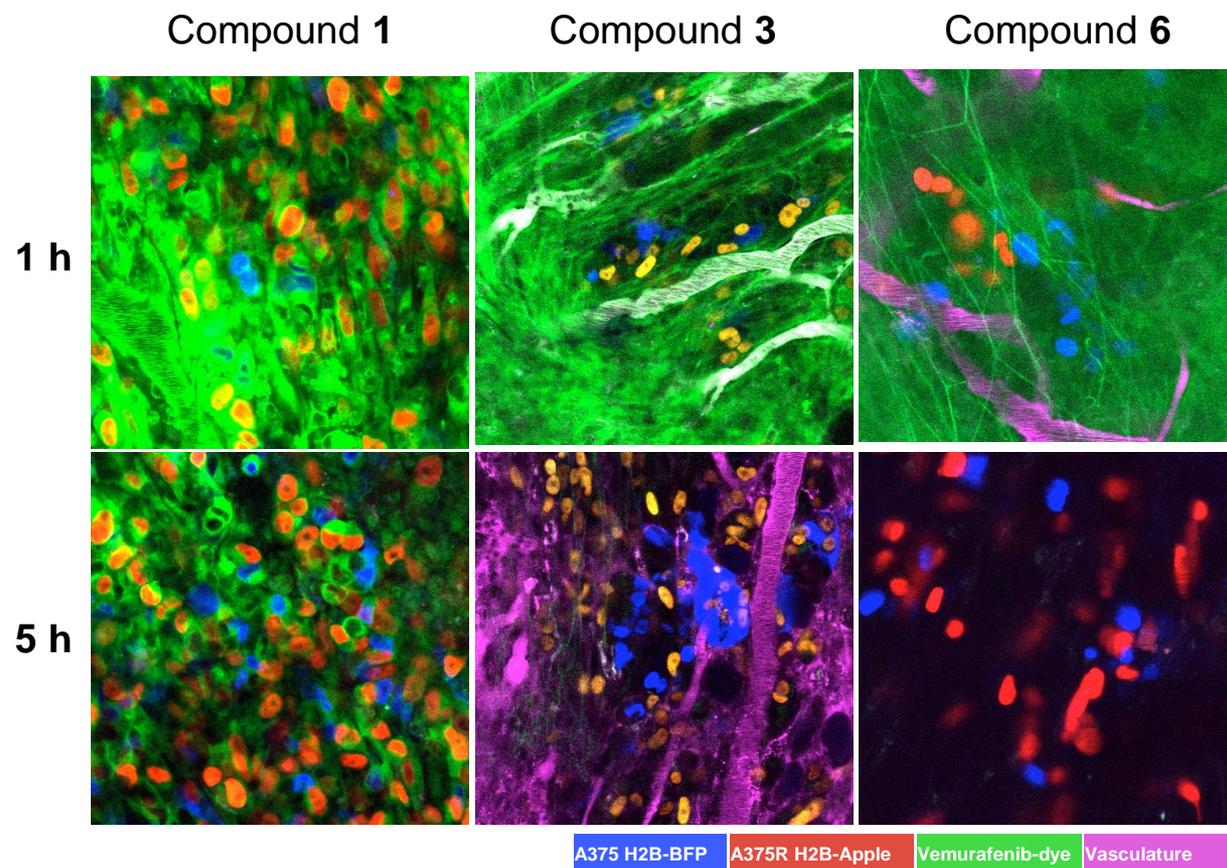
**Fig. S2.** Synthesis of fluorescent derivatives of vemurafenib.



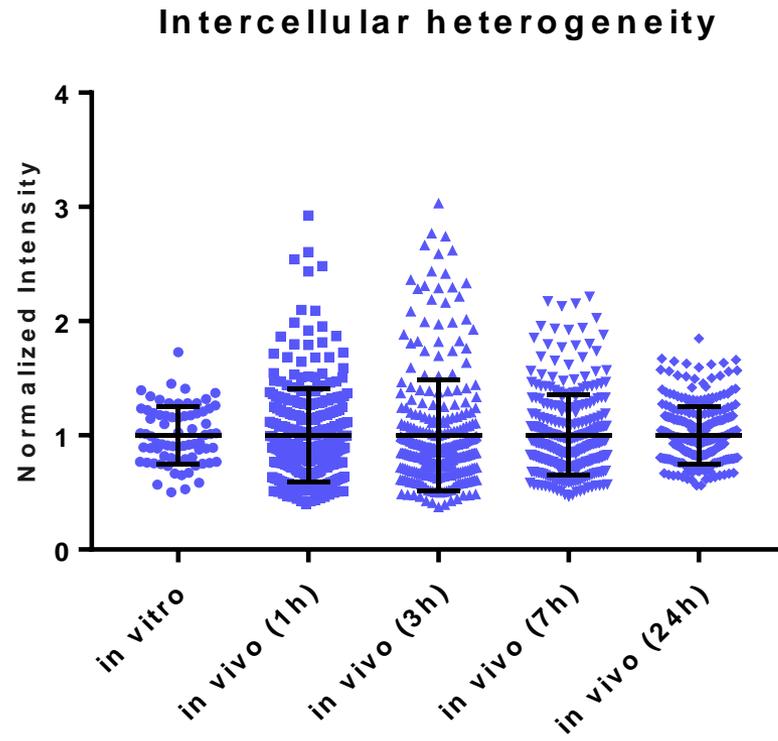
**Fig. S3.** Summary of the competitive imaging assay performed in non-resistant and resistant (R) melanoma cell lines, demonstrating the specificity of compound **1** (Vemurafenib-BODIPY).



**Fig. S4.** A schematic demonstrating the single-cell analysis of *in vivo* PK imaging of vemurafenib-BODIPY (1) made at a single region in a single mouse. A zStack was collected within the tumor and the nuclei of A375 H2B-BFP and A375R cells were segmented for each slice. The fluorescent intensity of the compound was measured in the cytoplasm of each cell in each slice. The intensity was normalized to baseline values.



**Fig. S5.** *In vivo* imaging reveals the superior pharmacokinetics and intracellular localization of compound 1 (Vemurafenib-BODIPY) in a melanoma tumor, whereas 3 and 6 are not retained in tumor cells.



**Figure S6.** Comparison of intercellular heterogeneity in vitro and in vivo at different time points (bars indicate mean $\pm$ SD; n>72).

**Table S1.** Summary of physical properties of all prepared fluorescent derivatives of vemurafenib.

Compound	Compound Number	MW (g/mol)	cLogP	Ex/Em (nm/nm)
Vemurafenib-BODIPY	<b>1</b>	772.63	7.0	504/516
Vemurafenib-Linker-BODIPY	<b>2</b>	917.78	5.9	
Vemurafenib-MayaFluor	<b>3</b>	871.31	5.6	491/504
Vemurafenib-Linker-MayaFluor	<b>4</b>	959.85	5.5	
Vemurafenib-SiR <sub>C</sub>	<b>5</b>	982.15	0.6	651/671
Vemurafenib-Linker-SiR <sub>C</sub>	<b>6</b>	1070.25	0.5	