Molsidomine Provides Neuroprotection Against Vincristine-induced Peripheral Neurotoxicity Through Soluble Guanylyl Cyclase Activation

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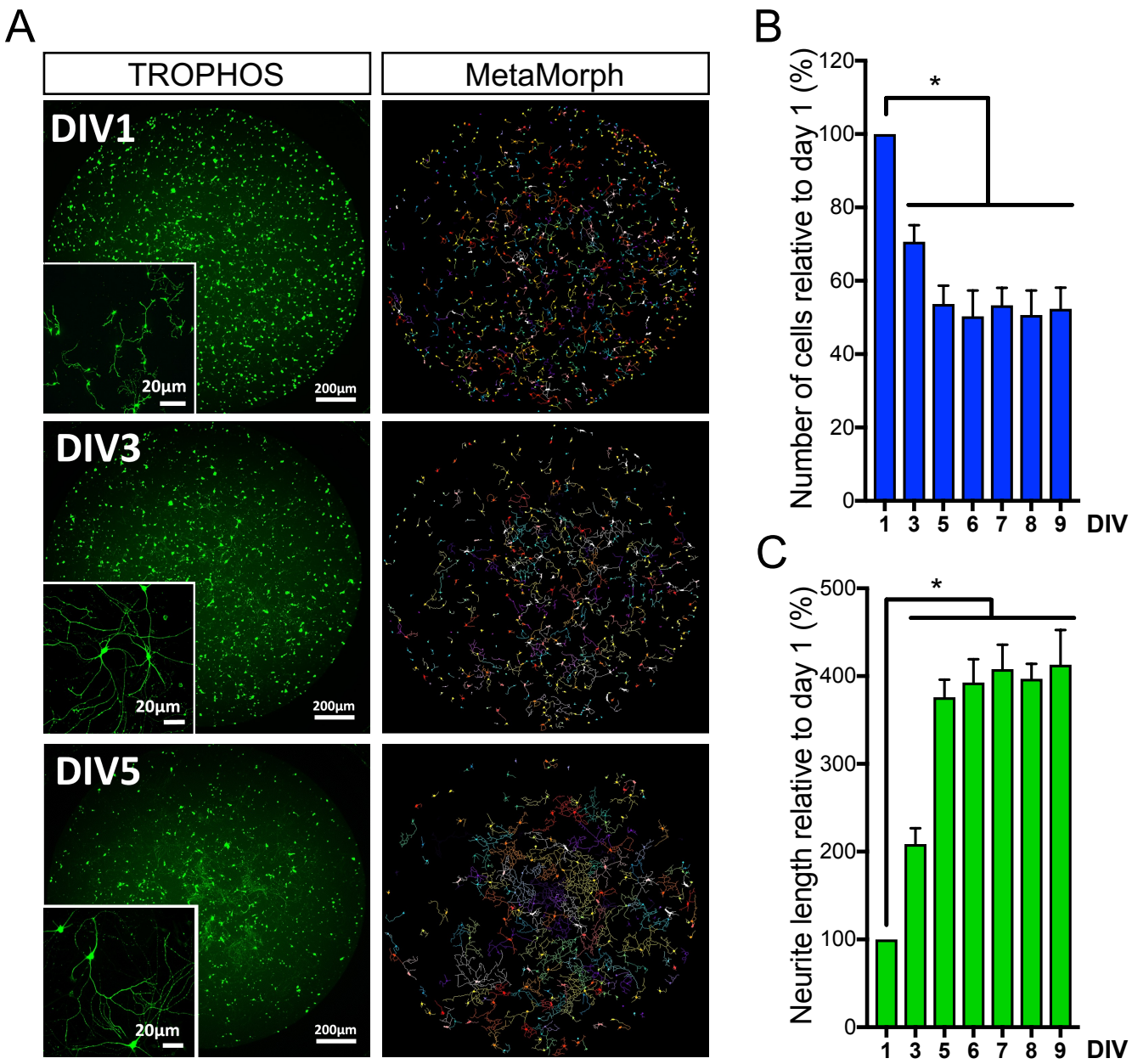
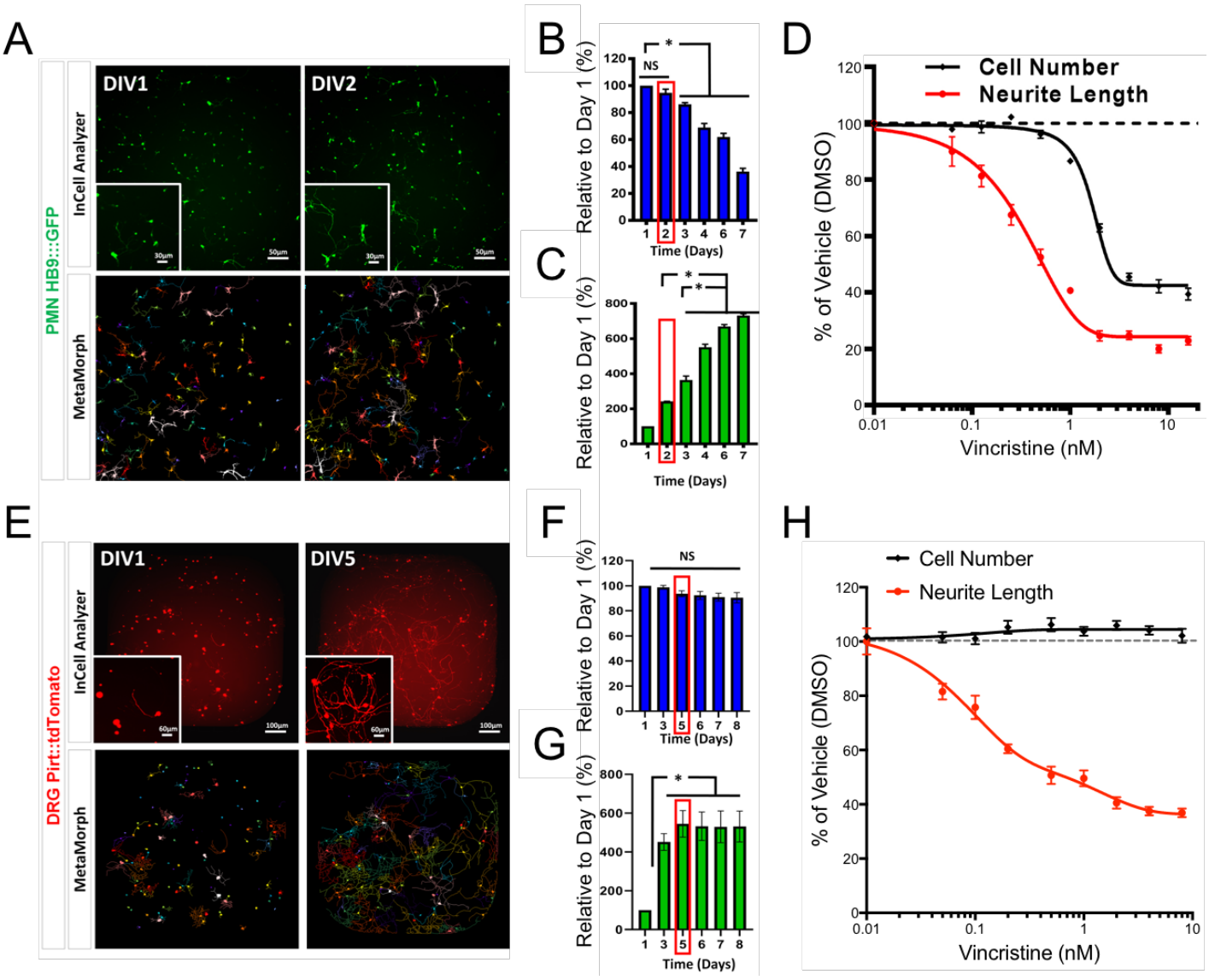
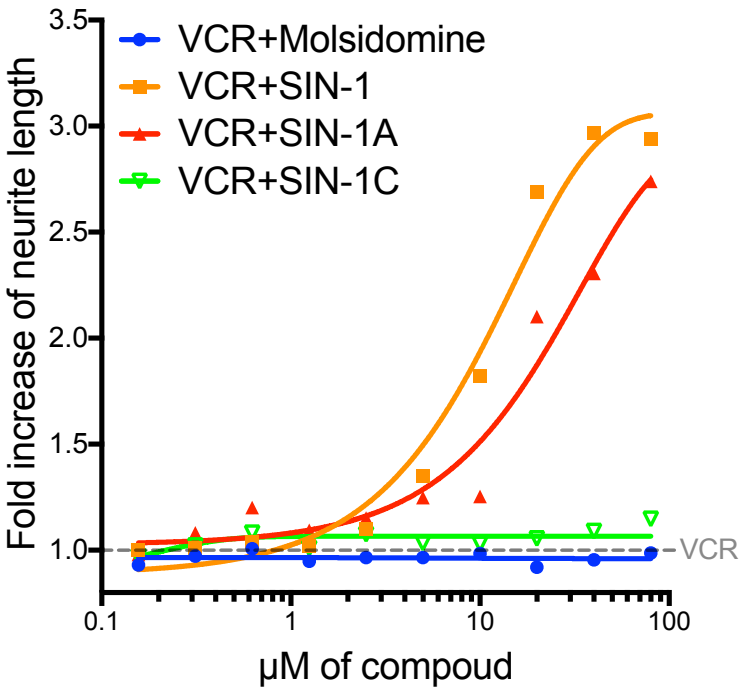


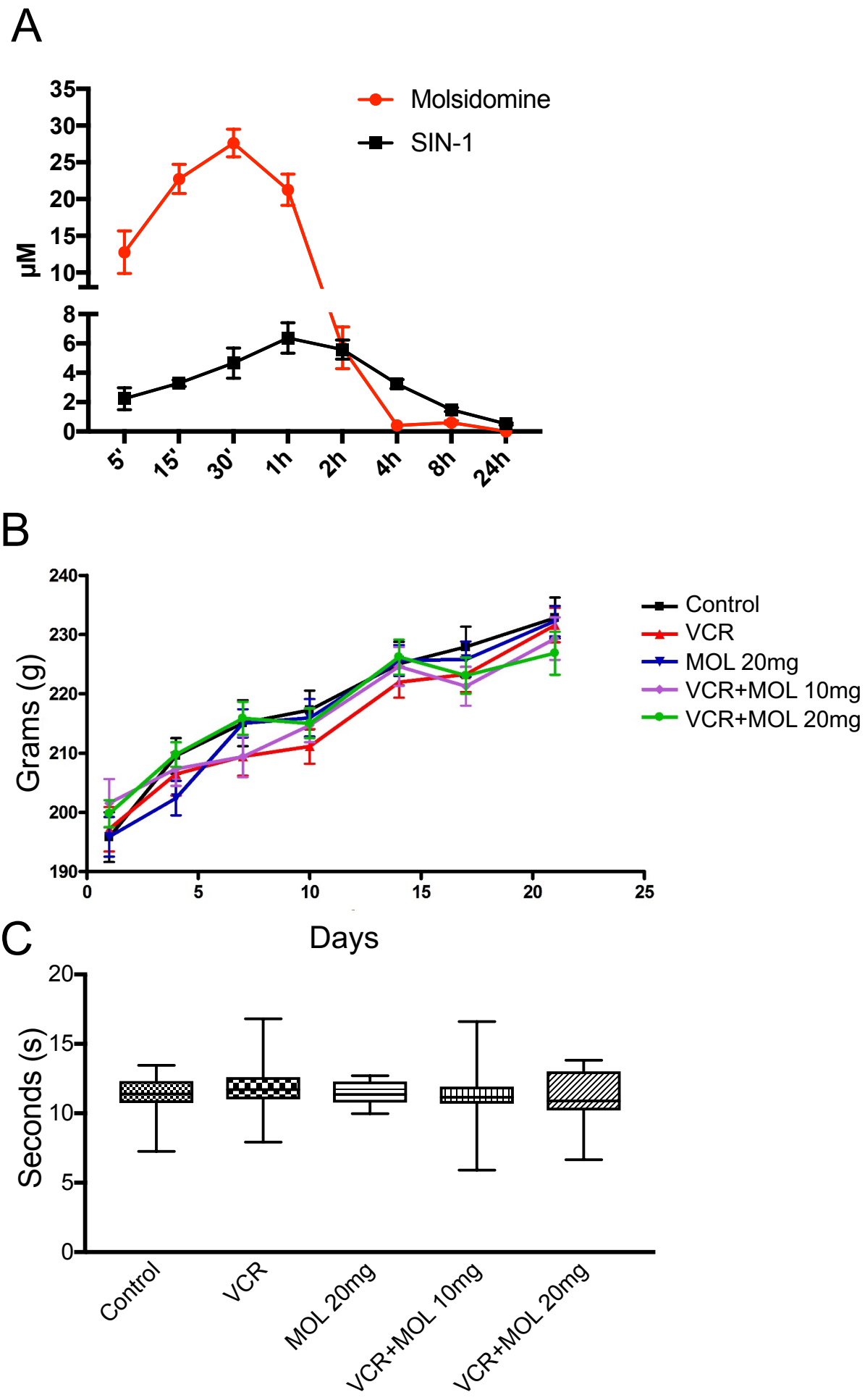
Supplementary Figure 1 (related to Fig. 1). A neuron-based model for the screening of agents preventing vincristine neurotoxicity. (A) Left panels – whole-well images of live GFP⁺ ES-MNs at DIV1, 3 and 5 obtained with Trophos (insets show enlarged images of neurons). Right panels – whole well images processed with MetaMorph for measurements of cellular quantity and neurite length in pseudo colors. Scale bar 200µm and 20µm in the insets (B and C) Kinetics of ES-MNs survival (B) and neurite growth (C) during 9 days *in vitro*. Data are presented as Mean ± SEM, n=4; One-way ANOVA, repeated measurements. *$p<0.0029$ in (B); *$p\leq0.001$ in (C).



Supplementary Figure 2 (related to Fig. 2) Cell-based assays for hits validation in primary motor and sensory neurons. (A) Top panels –images of live GFP+ pMNs at DIV1 and DIV2 obtained with InCell Analyzer in 384-well plates (insets show enlarged images of pMNs). Bottom panels –images processed with MetaMorph for measurements of cellular quantity and neurite length in pseudo colors. Scale bar, 50µm and 30µm in insets. (B and C) Kinetics of pMNs survival (B) and neurite growth (C) during 7 days *in vitro*. Red boxes indicate the selected timepoint for starting VCR treatment. Data are presented as Mean ± SEM, n=3; One-way ANOVA, repeated measurements. *p<0.05 in (B); *p≤0.001 in (C). (D) Dose-dependent effect of VCR on pMNs neurite length (red curve) and cell number (black curve). VCR doses are expressed in log scale. Data are presented as Mean ± SEM of 3 independent experiments. (E) Top panels – whole well images of alive td-Tomato+ DRG neurons at DIV1 and DIV5 obtained with InCell Analyzer in 384-well plates (insets show enlarged images of DRG neurons). Bottom panels – whole well images processed with MetaMorph for measurements of cellular quantity and neurite length in pseudo colors. Scale bar, 100µm and 60µm in insets. (F and G) Kinetics of DRG neurons survival (F) and neurite growth (G) during 8 days *in vitro*. Red boxes indicate the selected timepoint for starting VCR treatment. Data are presented as Mean ± SEM, n=3; One-way ANOVA, repeated measurements. *p<0.0001 in (F); *p≤0.0001 in (G). (H) Dose-dependent effect of VCR on DRG neurons neurite length (red curve) and cell number (black curve). VCR doses are expressed in log scale. Data are presented as Mean ± SEM of 3 independent experiments.



Supplementary Figure 3 (related to Fig. 3): The NO donor activity of SIN-1 is required for its neuroprotection . Dose-response curves for molsidomine derivatives on ES-MNs neurite length. SIN-1 and SIN-1A protect neurite length against VCR neurotoxicity. Doses of compounds are expressed in log scale. Values are means of six technical replicates; error bars are not shown for clarity.



Supplementary Figure 4 (related to Fig. 4): Chronic vincristine and molsidomine treatments are well tolerated. (A) Molsidomine (red line) and SIN-1 (black line) levels in plasma from rats treated with 20 mg/kg of molsidomine. Data are presented as mean ± SEM (n=5). (B) Body weight gain during the course of the treatment with vincristine (VCR) and molsidomine (MOL). Data are presented as mean ± SEM (n=12). (C) Effect of MOL treatment on VCR-induced thermal sensitivity. Noxious thermal stimulus (plantar analgesiometer test) doesn't show any significant changes after VCR administration and after MOL treatment. Data are presented as mean ± SEM (n=12).