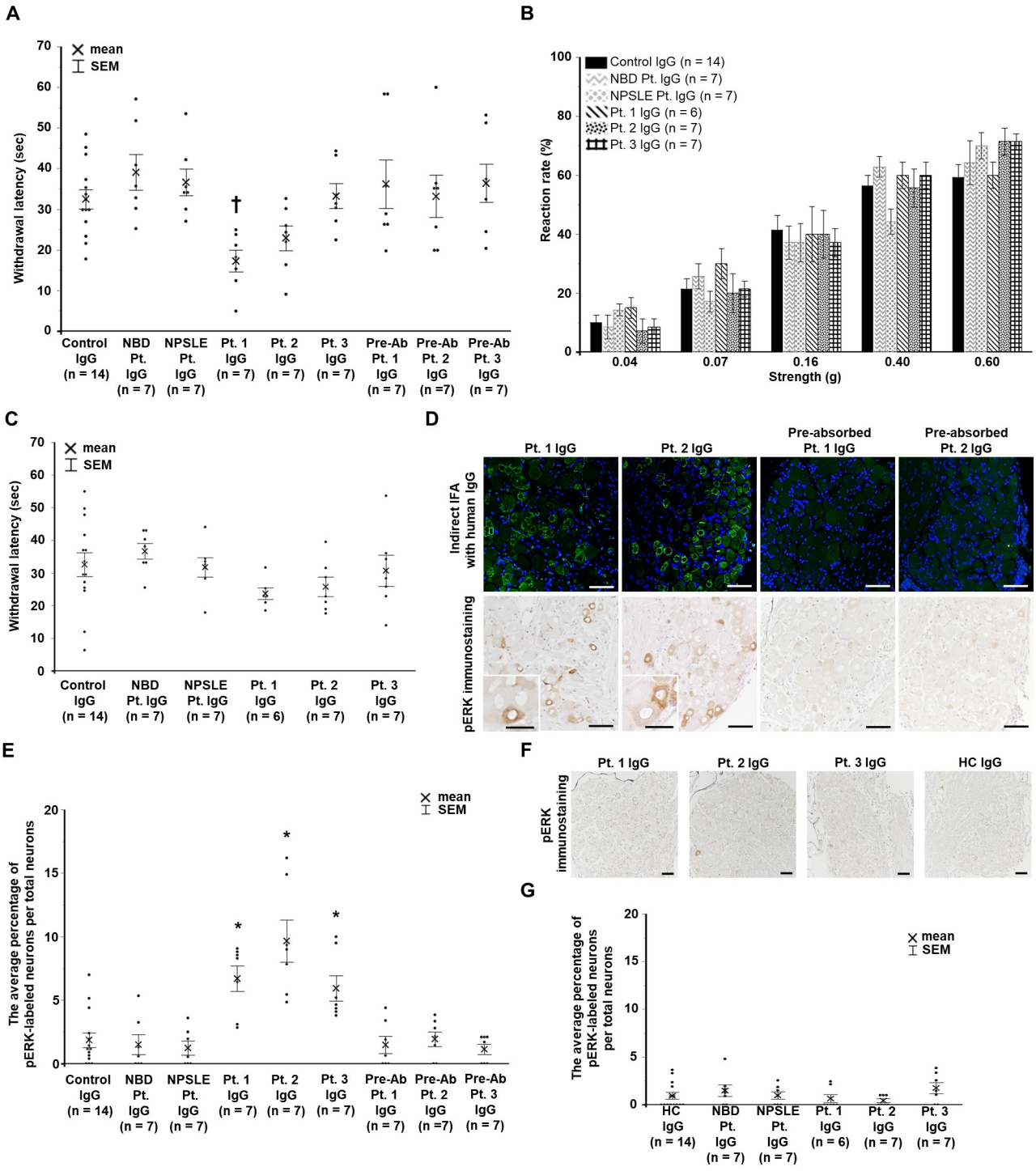


**figure e-2: Assessment of pain-like behaviors and neuronal activation in passive transfer mice 24 and 48 h after injection.**

**(A)** Thermal pain hypersensitivity was assessed 24 h after injection using a digital hot-plate set to 48° C. The withdrawal latency of mice treated with IgG from Patient 1 was significantly shorter than that of control IgG-treated mice (mean ± SEM; 17.2 ± 2.7 s and 32.5 ± 2.4 s, respectively: one-way ANOVA with Dunnett's test; †$p < 0.05$) while there were no significant differences in the withdrawal latencies between mice treated with control IgG and IgG from a NBD patient, a NPSLE patient, and Patients 2 and 3 (mean ± SEM; 32.5 ± 2.4 s, 39.1 ± 4.4 s, 36.6 ± 3.3 s, 22.9 ± 3.1 s, and 33.2 ± 3.0 s, respectively: one-way ANOVA with Dunnett's test). Again, no significant differences in the withdrawal latencies were noted between mice treated with control IgG and IgG from Patients 1, 2, and 3 after pre-adsorption with rhPlexin D1 (mean ± SEM; 32.5 ± 2.4 s, 36.2 ± 6.0 s, 33.2 ± 5.2 s, and 36.4 ± 4.7 s, respectively: one-way ANOVA with Dunnett's test). **(B)** Mechanical pain hypersensitivity was assessed by calibrated von Frey filaments (0.04, 0.07, 0.16, 0.40, and 0.60 g) 48 h after injection. There were no significant differences in the reaction rates to each stimulation strength between mice treated with control IgG and IgG from a NBD patient, a NPSLE patient, and Patients 1, 2, and 3 (one-way ANOVA with Dunnett's test). **(C)** Thermal pain hypersensitivity was assessed 48 h after injection using a digital hot-plate set to 48° C. No significant differences in the withdrawal latencies were observed between mice treated with control IgG and IgG from a NBD patient, a NPSLE patient, and Patients 1, 2, and 3 (mean ± SEM; 32.6 ± 3.6 s, 36.6 ± 2.4 s, 31.7 ± 2.9 s, 23.7 ± 1.8 s, 25.7 ± 3.0 s, and 30.7 ± 4.7 s, respectively: one-way ANOVA with Dunnett's test). **(D)** Indirect IFA of mouse L5 DRG sections revealed that IgG (green) from two patients with Plexin D1-IgG (Patients 1 and 2) bound to small DRG neurons. IgG from Patients 1 and 2 pre-absorbed with rhPlexin D1 (2 µg/ml) showed no significant immunoreactivity to mouse DRG. Nuclei are counterstained with 4′,6-diamidino-2-phenylindole (DAPI) (blue). Immunostaining of pERK, a marker of primary afferent neuron activation, in L5 DRG of mice treated with purified IgG from Patients 1 and 2 24 h after injection. Most of the pERK-labeled neurons in mice treated with purified IgG from Patients 1 and 2 are small DRG neurons (≤ 25 µm in diameter). Few neurons are labeled for pERK in mice treated with IgG from Patients 1 and 2 after pre-absorption with rhPlexin D1. Sections were counterstained with hematoxylin. **(E)** The percentages of pERK-labeled neurons among total DRG neurons in mice treated with purified IgG from Patients 1, 2, and 3 were significantly larger than that in mice treated with control IgG 24 h after injection (mean ± SEM; 6.7 ± 1.0%, 9.7 ± 1.7%, 5.9 ± 1.0%, 1.9 ± 0.6%, respectively: one-way ANOVA with Dunnett's test; *$p < 0.01$). IgG from a NBD patient, a NPSLE patient, and Patients 1, 2, and 3 pre-absorbed with rhPlexin D1 showed no significant changes in pERK-labeled neuron percentages compared with control IgG (mean ± SEM; 1.5 ± 0.8%, 1.2 ± 0.6%, 1.5 ± 0.7%, 1.9 ± 0.6%, 1.1 ± 0.4%, 1.9 ± 0.6%, respectively: one-way ANOVA with Dunnett's test). **(F)** Forty-eight hours after injection, few DRG neurons were labeled with pERK in mice treated with control IgG or IgG from Patients 1, 2, and 3. Sections were counterstained with hematoxylin. **(G)** Forty-eight hours after injection, no significant change in percentages of pERK-labeled DRG neurons was found between control IgG and IgG from a NBD patient, a NPSLE patient, and Patients 1, 2, and 3 (mean ± SEM; 0.9 ± 0.4%, 1.5 ± 0.6%, 1.0 ± 0.4%, 0.6 ± 0.4%, 0.4 ± 0.2%, and 1.8 ± 0.6%, respectively: one-way ANOVA with Dunnett's test).

Scale bars = 50 µm and (inset) = 25 µm. ANOVA = one-way analysis of variance; DRG = dorsal root ganglia; HC = healthy control; IFA = immunofluorescence assay; IgG = immunoglobulin G; NBD = neuro-Behçet's disease; NPSLE = neuropsychiatric systemic lupus erythematosus; pERK = phosphorylated extracellular signal-regulated protein kinase; rhPlexin D1 = recombinant human Plexin D1; SEM = standard error of the mean.