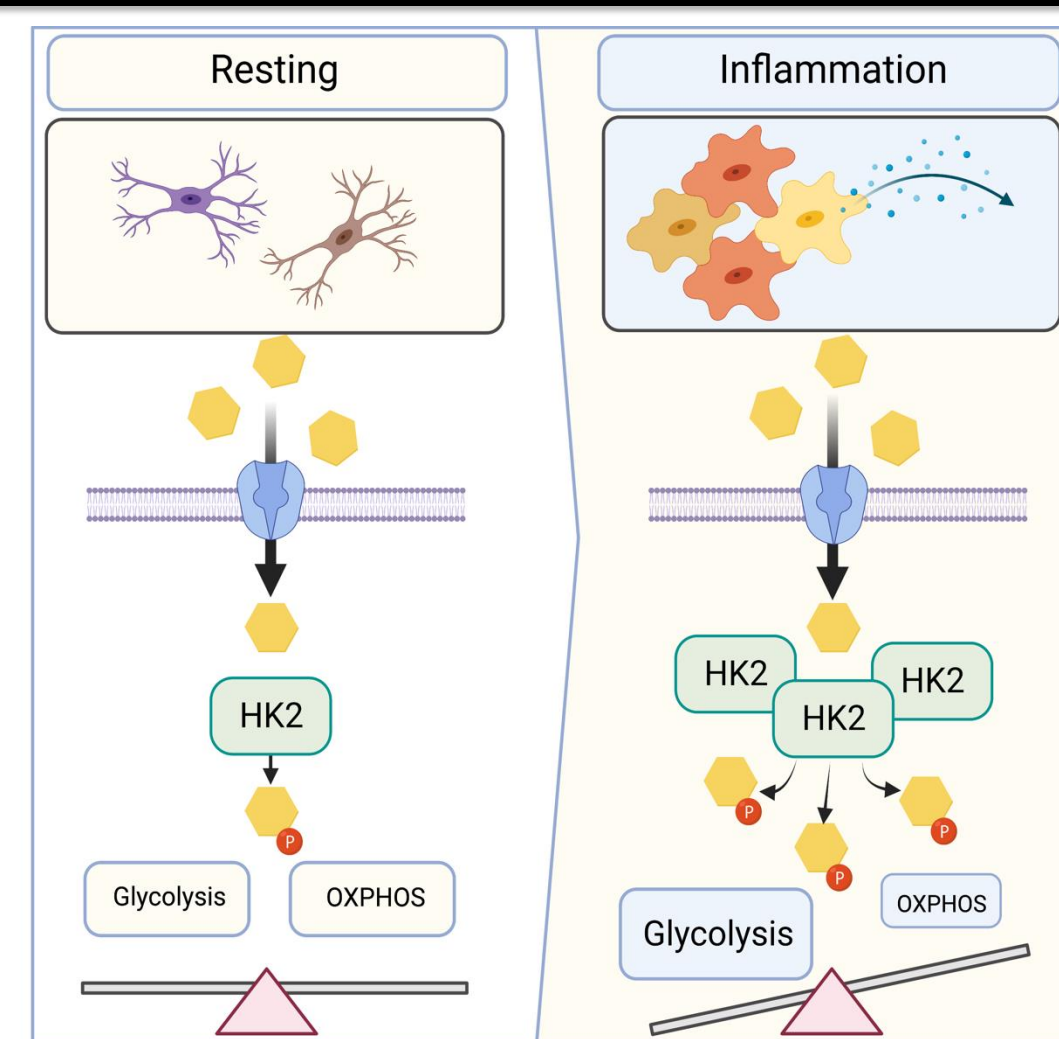


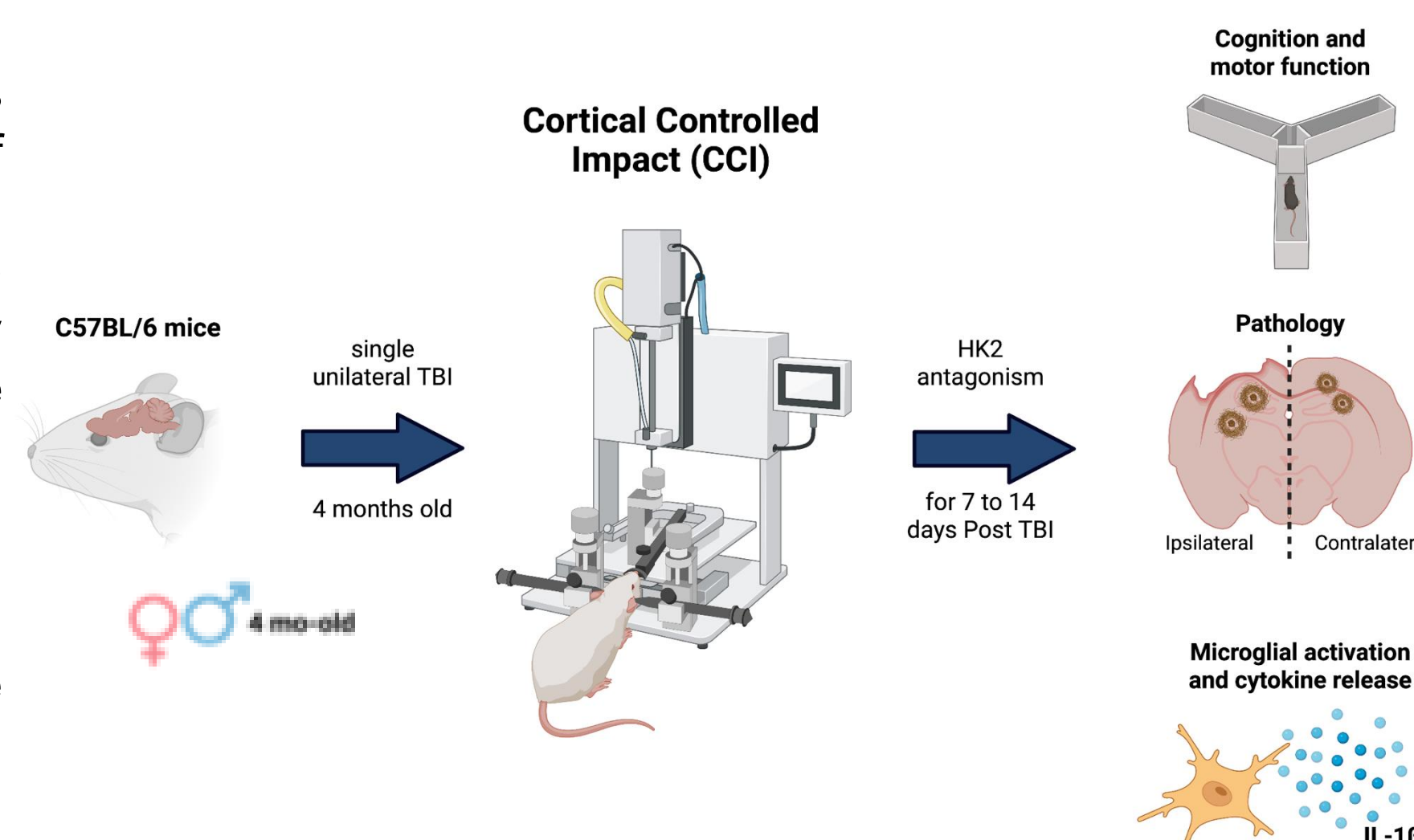
Pharmacological inhibition of HK2 improves motor function after TBI

TBI causes an immediate primary injury resulting from mechanical tissue damage. This is followed by a prolonged secondary injury phase characterized by neuroinflammation, cellular dysfunction, and progressive tissue damage that can contribute to long-term neurological impairment. Microglia are key mediators of this secondary inflammatory response and undergo metabolic adaptations that support inflammatory signaling. Hexokinase 2 (HK2), a glycolytic enzyme implicated in regulating microglial inflammatory activity, has emerged as a potential therapeutic target in neurological disease; however, its role following TBI remains poorly understood. We therefore investigated whether pharmacological and genetic inhibition of HK2 attenuates post-traumatic neuroinflammation and improves functional recovery after experimental TBI.



Methods

Figure 1. 4-month-old C57BL/6 mice underwent open-head controlled cortical impact (CCI) to TBI. Beginning 24 hours post-injury, mice received daily intraperitoneal injections of the HK2 inhibitor lonidamine (LND) for 7 consecutive days and were analyzed 15 days after injury. Motor performance was assessed using the rotarod test, and neuroinflammatory responses were evaluated in cortical and hippocampal tissue using quantitative PCR, immunohistochemistry, and immunoblotting. To determine whether these effects were mediated by microglial HK2, complementary studies were performed using mice with partial genetic deletion of HK2 in microglia. For all graphs, individual data points from female and male mice are indicated by pink and black symbols, respectively.



TBI induces HK2 expression in the injured brain and IBA1⁺ cells

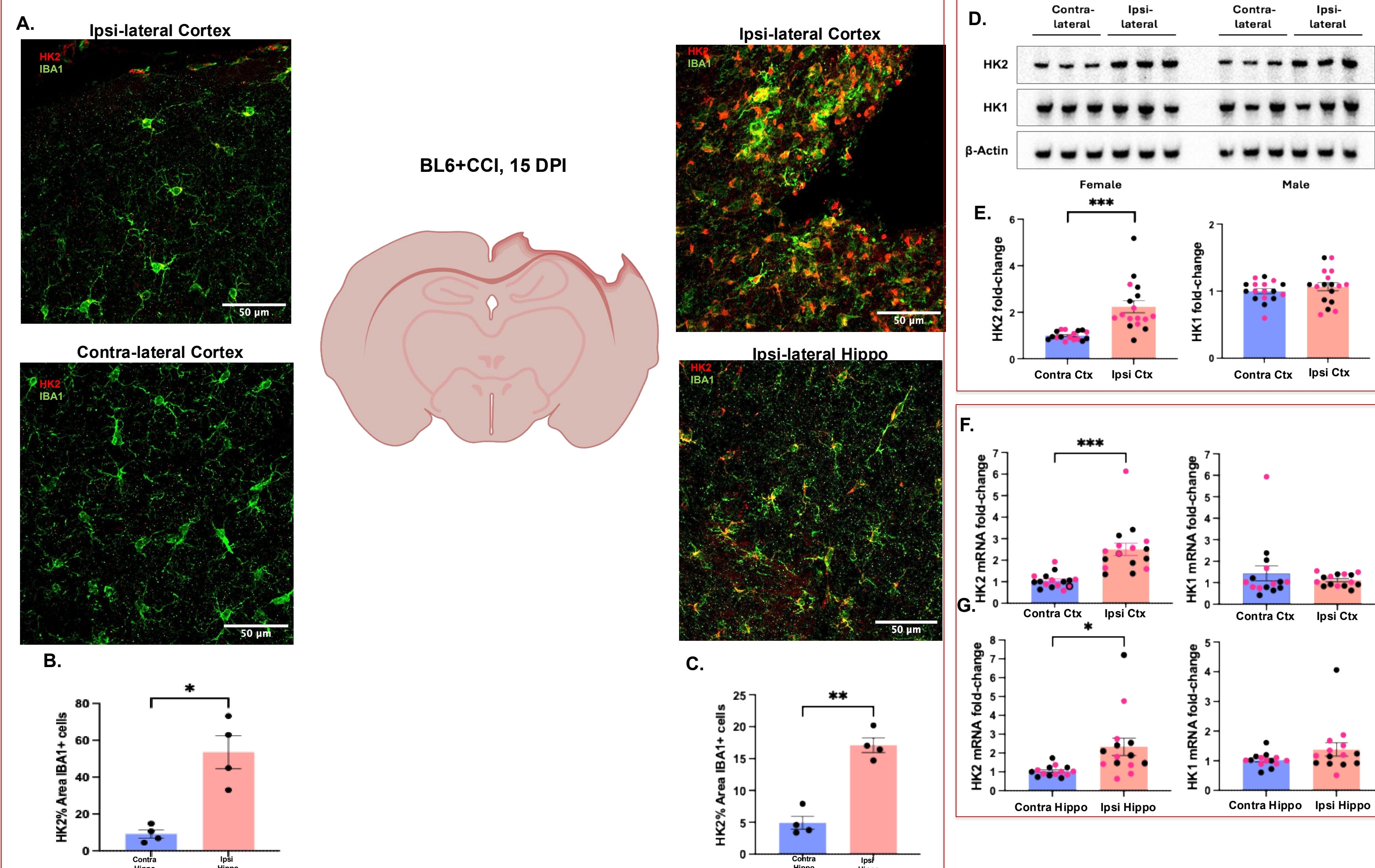


Figure 2. (A) Representative images of HK2 expression in IBA1⁺ microglia within the ipsilateral and contralateral cortex and hippocampus 15 days after TBI. **(B)** Increased HK2 immunoreactivity is observed in the ipsilateral cortex adjacent to the injury site and in the **(C)** ipsilateral hippocampus with respect to its contralateral side (n = 4). **(D-E)**. Representative immunoblot of HK1 and HK2 protein levels in ipsilateral and contralateral cortical tissue 15 days after TBI. While HK1, the predominant neuronal hexokinase isoform, remained unchanged, HK2 expression was increased in the injured hemisphere. **(F-G)** Relative Hk1 and Hk2 mRNA expression in cortical and hippocampal tissue measured by qPCR. (n = 9 females and n=8 males, *p < 0.05 and ***p < 0.001, paired T test)

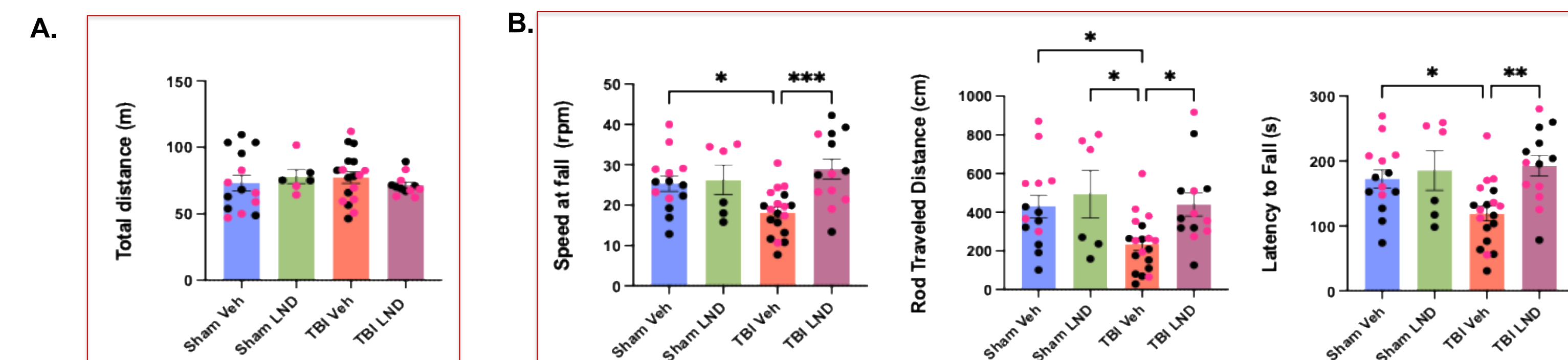


Figure 3. (A) Locomotor activity was assessed by open field total distance traveled. **(B)** Motor performance was evaluated using the rotarod test, including maximal speed, distance traveled, and latency to fall. Data were analyzed using one-way ANOVA followed by Tukey's multiple comparisons test (n = 6–13 per group). Statistical significance was defined as *p < 0.05, **p < 0.01, and ***p < 0.001.

HK2 inhibition attenuates hippocampal microglial activation and inflammasome-associated gene expression

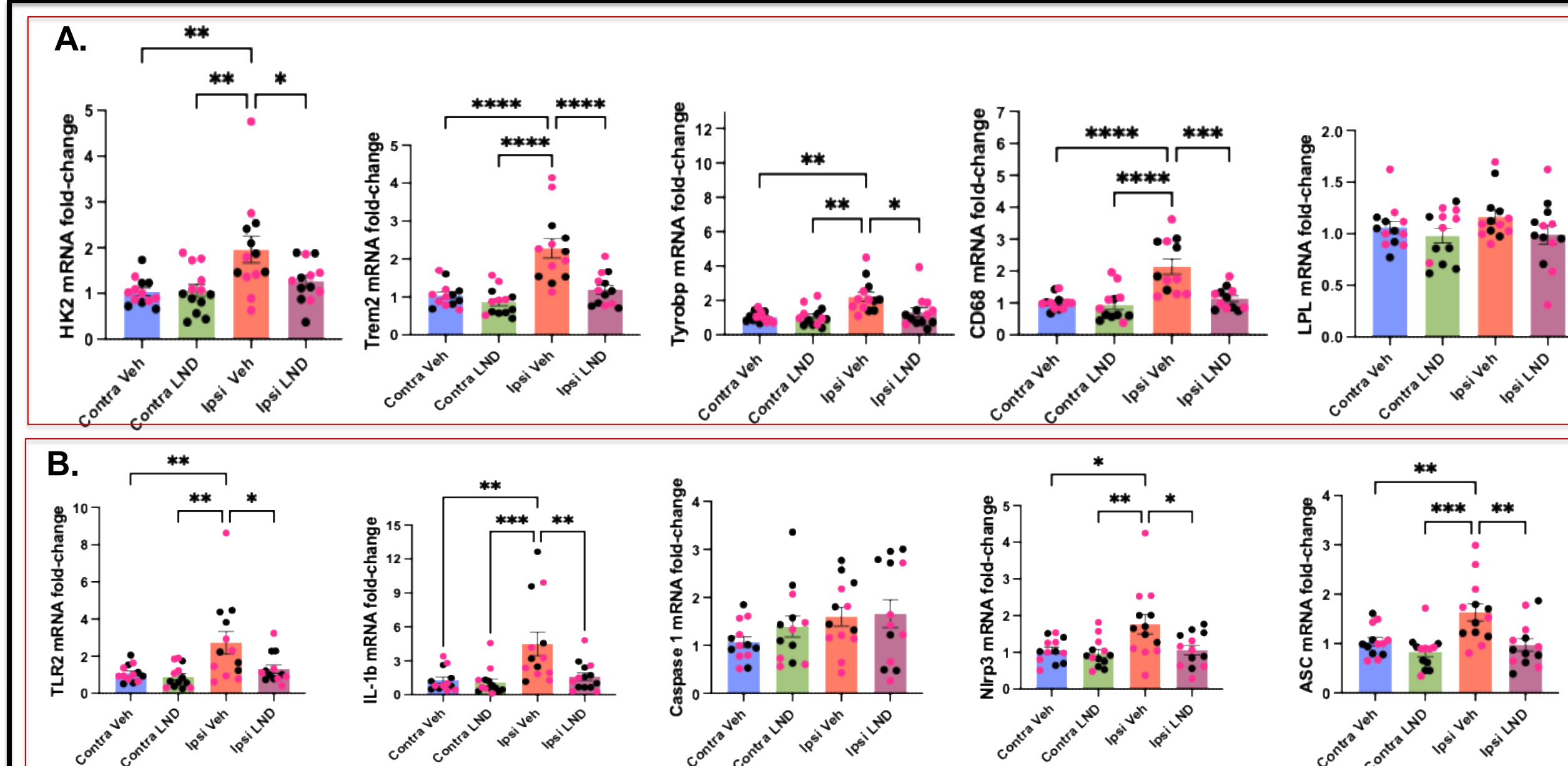


Figure 4. Relative mRNA expression of microglial activation-associated genes **(A)** and inflammasome-related genes **(B)** in contralateral and ipsilateral hippocampal tissue collected 15 days after TBI, as determined by qPCR. Data were analyzed using one-way ANOVA followed by Tukey's multiple comparisons test. Statistical significance was defined as * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, and **** $p < 0.0001$.

HK2 antagonism reduces microglial ASC accumulation in the injured cortex and hippocampal hilus after TBI

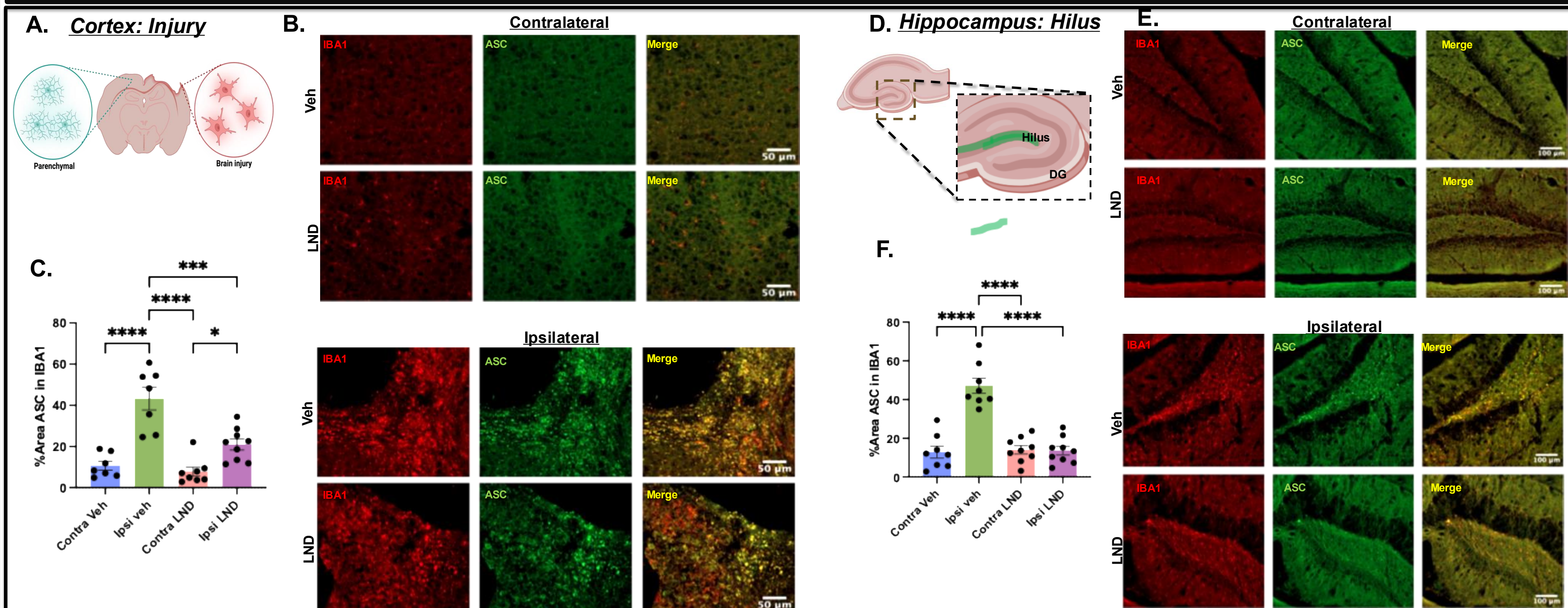


Figure 5. (A–B) Schematic and representative immunofluorescence images of IBA1 and ASC in the ipsilateral injured cortex and contralateral cortex. **(C)** Quantification of ASC immunoreactivity normalized to IBA1⁺ area demonstrates increased ASC accumulation after CCI that is reduced by LND treatment. (*n* = 7–8 per group, **p* < 0.05, ****p* < 0.001, and *****p* < 0.0001, Ordinary one-way ANOVA with post hoc Tukey's multiple comparisons) **(D–E)** Schematic and representative images of the hippocampal dentate gyrus highlighting ASC accumulation in IBA1⁺ microglia within the ipsilateral hilus. **(F)** Quantification shows robust ASC induction in the ipsilateral hilus following CCI, which is markedly reduced by LND treatment to levels comparable to contralateral controls. (*n* = 7–8 per group, *****p* < 0.0001, Ordinary one-way ANOVA with post hoc Tukey's multiple comparisons)

Conclusions

1. TBI induces sustained microglial HK2 expression in cortical and hippocampal regions vulnerable to secondary injury.
2. Pharmacological inhibition of HK2 improves motor performance and attenuates hippocampal inflammatory responses following TBI.
3. Microglial HK2 represents a promising immunometabolic target for limiting secondary neuroinflammation after TBI.

References and Acknowledgments

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Questions or collaborations?

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