Voltage-activated human ether-à-go-go–related gene (hERG) potassium channels are critical for the repolarization of cardiac action potentials and tune-spike frequency adaptation in neurons. Two isoforms of mammalian ERG1 channel subunits, ERG1a and ERG1b, are the principal subunits that conduct the I_{Kr} current in the heart and are also broadly expressed in the nervous system. However, there is little direct evidence that ERG1a and ERG1b form heteromeric channels. Here, using electrophysiology, biochemistry, and fluorescence approaches, we systematically tested for direct interactions between hERG1a and hERG1b subunits. We report 1) that hERG1a dominant-negative subunits suppress hERG1b currents (and vice versa), 2) that disulfide bonds form between single cysteine residues experimentally introduced into an extracellular loop of hERG1a and hERG1b subunits and produce hERG1a–hERG1b dimers, and 3) that hERG1a and hERG1b subunits tagged with fluorescent proteins that are FRET pairs exhibit robust energy transfer at the plasma membrane. Thus, multiple lines of evidence indicated a physical interaction between hERG1a and hERG1b, consistent with them forming heteromeric channels. Moreover, co-expression of variable ratios of hERG1a and hERG1b RNA yielded channels with deactivation kinetics that reached a plateau and were different from those of hERG1b channels, consistent with a preference of hERG1b subunits for hERG1a subunits. Cross-linking studies revealed that an equal input of hERG1a and hERG1b yields more hERG1a–hERG1a or hERG1a–hERG1b dimers than hERG1b–hERG1b dimers, also suggesting that hERG1b preferentially interacts with hERG1a. We conclude that hERG1b preferentially forms heteromeric ion channels with hERG1a at the plasma membrane.

ERG K⁺ channels are expressed in the heart (1, 2), central nervous system (3), and in a variety of other tissues, including tumor cells (4, 5). The disease relevance of human ether-à-go-go–related gene (hERG)² in the heart is underscored by inherited mutations in the hERG gene that cause Type 2 long QT syndrome, a cardiac arrhythmia that is characterized by prolonged action potentials that can lead to sudden death (1). Furthermore, the inhibition of hERG channels by numerous pharmaceuticals accounts for a common acquired form of LQTS (6). In mammalian heart, ERG1a and ERG1b isoforms are the principal (α) subunits that form the cardiac I_{Kr} current (2, 6–10) whose role is to repolarize action potentials (11, 12). ERG1a and ERG1b differ in their primary structure and functional properties. ERG1a channels have a long N-terminal region that contains a regulatory “eag” domain (6, 10, 13), whereas ERG1b channels have a novel, short N-terminal region and lack an eag domain (2, 9) (Fig. 1A). Under heterologous expression, ERG1a forms homotetrameric channels (Fig. 1A), which have robust currents and slow kinetics of channel closing (6, 10), whereas ERG1b forms homotetrameric channels (Fig. 1A) with small currents and kinetics of closing that are 5–10-fold faster than those of ERG1a channels (2, 9). Fast deactivation of ERG1b is due to the lack of the eag domain, which promotes slow deactivation in ERG1a (14). Co-expression of mammalian ERG1a and ERG1b (2, 15) leads to currents with kinetics similar to that of I_{Kr} in adult mammalian cardiomyocytes (11, 12) and cardiomyocytes derived from human-induced pluripotent stem cells (hiPSC-CMs) (7). Genetic mutations in both human ERG1a and ERG1b subunits are linked to LQT2 (1, 15, 16) indicating that the pathophysiological importance of both isoforms.

hERG1a and hERG1b are clearly both critical to form I_{Kr}, and there is some evidence that the subunits co-associate. ERG1a and ERG1b isoforms co-immunoprecipitate in tumor cells, brain, and cardiomyocytes (3, 4, 8, 17) consistent with subunit interactions. Co-expression of ERG1a and ERG1b subunits in Xenopus oocytes led to currents with a time course of deactivation that was not explained by algebraic summation and weighting of currents from homomeric channels, but rather was more consistent with an interaction between ERG1a and ERG1b subunits (2). An N-terminal domain of hERG1a channels interacts with the N-terminal domain of hERG1b channels in protein-interaction assays, which is also consistent with the idea that the full-length hERG1a and hERG1b channels interact (18). Drugs inhibit currents differently in HEK293 cells expressing hERG1a versus hERG1a and hERG1b (19) indicating that the composition of cardiac I_{Kr} may be critical for acquired LQTS. In summary, both mammalian ERG1a and ERG1b are critically important molecular constituents of cardiac I_{Kr} and play a role in human pathophysiology, but more robust evidence and more direct methods need to be established to deter-

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3 The abbreviations used are: hERG, human ether-à-go-go–related gene; eCFP, enhanced cyan fluorescent protein.
mine the putative interaction and multimerization of hERG1a and hERG1b subunits.

In this study, we tested for direct, physical interactions between hERG1a and hERG1b subunits using electrophysiological, biochemical, and optical methods. We found that dominant-negative hERG1b subunits suppressed currents from hERG1a (and vice versa), indicating direct hERG1a and hERG1b subunit interactions. Using disulfide bond formation between a cysteine residue introduced into the extracellular loop between the S5 and P-loop domains of hERG1a subunits (L589C) and the equivalent site in hERG1b subunits (L249C), we found robust disulfide bonds between hERG1a and hERG1b subunits, indicating that these subunits were in close proximity. Using Förster resonance energy transfer (FRET) and CFP/Citrine fluorescent protein FRET pairs fused to hERG1a and hERG1b, we found robust FRET between hERG1a-CFP and hERG1b-Citrine and hERG1a-Citrine and hERG1b-CFP, indicating close physical proximity between hERG1a and hERG1b subunits at the plasma membrane. Our results strongly suggest that hERG1a and hERG1b subunits form heteromeric hERG1a/hERG1b channels at the plasma membrane. Moreover, cross-linking experiments showed that more hERG1a–hERG1b dimers were formed than hERG1b–hERG1b dimers when equivalent amounts of hERG1a and hERG1b were co-expressed. This result suggests that hERG1b prefers to interact with hERG1a instead of hERG1b.

Results

Functional co-expression of hERG1a and hERG1b subunits

To determine the functional properties of hERG1a subunits in the presence of hERG1b subunits (Fig. 1A), we co-expressed hERG1a with various ratios of hERG1b RNA in Xenopus oocytes and performed two-electrode voltage-clamp recordings. Expressed alone, hERG1a has robust currents with characteristic slow closing (deactivation) kinetics (Fig. 1, B–D). In contrast to hERG1a, hERG1b has very small currents and faster deactivation kinetics (Fig. 1, B–D). Using different mixtures of hERG1a and hERG1b subunit cRNA ratios we found that the kinetics of deactivation became faster with increasing amounts of hERG1b cRNA (Fig. 1, B–D). However, the deactivation kinetics in hERG1a and hERG1b subunit mixtures did not reach the very fast kinetics measured for homomeric hERG1b channels, but instead reached a limit at RNA ratios of hERG1a to hERG1b of 1:4 or 1:8 and 1:16 (Fig. 1, C and D). Because a limit was reached, instead of the kinetics becoming similar to those of homomeric hERG1b, we interpret this result to mean that there was likely not an excess of homomeric hERG1b channels at the plasma membrane. Instead, we propose that the results are consistent with formation of hERG1a/hERG1b heteromeric channels. The functional limit is consistent with the idea that hERG1b subunits preferentially interact with hERG1a subunits.

Dominant-negative hERG1b G288S subunits suppress hERG1a currents

To test for functional interactions between hERG1a and hERG1b we used hERG1a G628S subunits or their equivalent in hERG1b, hERG1b G288S (Fig. 2A), as tools to infer interaction with wild-type (WT) subunits. hERG1a G628S is expressed at the plasma membrane but does not have measurable K+ currents and co-expression of hERG1a G628S with WT hERG1a markedly reduces the WT current as if the hERG1a G628S mutant is a “poison subunit” that assembles with WT subunits and suppresses their function (20). Here, in a control experiment, hERG1a produced robust currents, hERG1a G628S did not produce measurable currents and a mixture of hERG1a and hERG1a G628S markedly reduced hERG1a current, as anticipated (Fig. 2, B, C, and E) and likewise, hERG1b produced current, hERG1b G228S did not have measurable currents, and hERG1b + hERG1b G228S did not have measurable ionic currents (Fig. 2, B, D, and E). We co-expressed hERG1a and hERG1b G228S at a 1:1 ratio and did not detect measurable K+ currents (Fig. 2, B, C, and E). We co-expressed hERG1b with hERG1a G628S at a 1:1 ratio and did not detect measurable K+ currents (Fig. 2, B, D, and E). These results show that hERG1a G628S suppressed the function of hERG1b channels and hERG1b G228S suppressed the function of hERG1a channels and indicates that hERG1a and hERG1b subunits interact. Because hERG1a G628S is expressed at the cell surface, we propose that non-functional heteromeric hERG1a/hERG1b channels are likely at the cell surface.

**hERG1a subunits make a disulfide bond with hERG1b subunits to form hERG1a–hERG1b dimers**

We examined potential direct and preferential subunit interactions between hERG1a and hERG1b subunits by introducing cysteine residues at a position at an extracellular site (Leu-589) in the S5–P region linker of hERG1a and the equivalent site in hERG1b subunits (L249C) and examining disulfide bond formation (Fig. 3A). As a positive control for dimer formation, hERG1a L589C subunits were expressed in Xenopus oocytes and purified from cell membranes, separated with SDS-PAGE, and analyzed with immunoblotting using anti-hERG-KA antibody, which equally recognizes hERG1a and hERG1b subunits (8) (see “Experimental procedures”). As shown previously (21), hERG1a L589C subunits formed two bands on a Western blot, one at the predicted size (130 kDa) of hERG1a subunit monomers and a second band at the expected size (260 kDa) of hERG1a–hERG1a dimers (Fig. 3B, lane 1), suggesting that hERG1a L589C subunits formed dimers because of spontaneous disulfide bonds. Likewise, hERG1b L249C subunits had a band on a Western blot that was of the predicted size (90 kDa) for hERG1b monomers and a band at the predicted size (180 kDa) for hERG1b–hERG1b dimers (Fig. 3B, lane 2). To test for a direct interaction between hERG1a and hERG1b subunits we co-injected oocytes with hERG1a L589C and hERG1b L589C subunits. In addition to bands corresponding to hERG1a monomers and dimers, and hERG1b monomers and dimers, Western blot analysis showed a new band at the predicted size (220 kDa) for hERG1a-hERG1b dimers (Fig. 3B, lane 3, red arrow), suggesting that hERG1a and hERG1b subunits were linked by a disulfide bond between L589C in hERG1a and L249C in hERG1b. As a negative control, we expressed hERG1a and hERG1b in separate oocytes, then mixed the oocytes during protein purification. Under these conditions, we detected bands corresponding to hERG1a monomers, hERG1a–hERG1a dimers, hERG1b monomers, and...
Figure 1. Coexpression of hERG1a and hERG1b subunits. A, schematic of hERG1a and hERG1b subunits. The PAS and CNBHD domains are indicated. The unique hERG1b region is denoted by the red part of the N-terminal region. Schematic of homotetrameric hERG1a or hERG1b channels showing two (of four) N-terminal domains for clarity. B, two-electrode voltage-clamp recordings of hERG1a, hERG1a plus hERG1b at a ratio of 1:1, 1:2, 1:4, 1:8, 1:16, and hERG1b. Scale bars are 2 μA and 1 s for all except hERG1b, which is 0.5 μA and 1 s. Voltage protocol is from a holding voltage of −80 mV with steps from −100 to 60 mV with repolarization to −50 mV. C and D, scatter plots of time constants (τ) derived from double-exponential fits (red lines) to the relaxation (deactivation) of the currents at −50 mV in B following a step to 60 mV. Horizontal lines are the mean and whickers are the S.D. * indicates p < 0.01 by one-way analysis of variance.
hERG1b–hERG1b dimers but, importantly, we did not detect a band corresponding to the size of hERG1a–hERG1b dimers (Fig. 3B, lane 4). Together the results of lanes 3 and 4 show that hERG1a–hERG1b dimers did not form merely as a side effect of membrane purification. Instead, we propose that hERG1a–hERG1b dimers were formed as a consequence of hERG1a and hERG1b assembly into heteromeric hERG1a/hERG1b channels.

We found that the predicted molecular weights for all dimer bands fit well with the experimental results, strongly suggesting that hERG subunits with introduced cysteines were not forming disulfides with an unidentified protein of a similar size, but hERG1b–hERG1b dimers.
rather hERG subunits formed dimers with other hERG subunits. We performed purifications in the presence of \( \beta \)-mercaptoethanol, a reducing agent, which abolished the bands at the predicted sizes for dimers, suggesting that the dimers were formed by disulfide bonds between introduced cysteines (Fig. 3C, lanes 5–7). Together, these results suggested that specific disulfide bonds were formed between hERG1a–hERG1a, hERG1b–hERG1b, or hERG1a–hERG1b subunits and that hERG1a and hERG1b subunits were within close enough proximity to form disulfide bonds. For the reactive –SH groups in cysteines to react, residues must be within 3.4–4.6 Å of one another (22) but disulfides have been reported to form between cysteines that are as much as 15 Å apart in flexible proteins (22, 23). The turret domain of hERG channels may be somewhat flexible (21) thus, the L589C and L249C sites, which are located in the turret, may be between 3.4 and 15 Å apart.

**hERG1b prefers to form disulfide bonds with hERG1a**

To examine disulfide bond formation between hERG1a L589C and hERG1b L249C in more detail, we expressed different amounts of hERG1a L589C relative to hERG1b L249C. First we expressed equivalent amounts of hERG1a L589C or hERG1b L589C proteins, as detected by Western blot analysis (Fig. 4, lanes 1 and 2). We then co-injected oocytes with hERG1a L589C and hERG1b L249C at 2:1, 1:1, and 1:2 ratios. We found that robust hERG1a–hERG1b dimers were formed at each ratio of input subunits (Fig. 4, lanes 3–5). We also detected relatively less hERG1b–hERG1b dimers compared with hERG1a–hERG1a dimers or hERG1a–hERG1b dimers at input ratios of hERG1a to hERG1b of 2:1 or 1:1 (Fig. 4, lanes 3 and 4). Thus, when the input amounts of hERG1a to hERG1b were at a 1:1 ratio there was less measurable hERG1b–hERG1b dimer than hERG1a–hERG1a or hERG1a–hERG1b dimers. We interpret our findings to mean that hERG1b subunits preferentially interact with hERG1a subunits. As ERG1a and ERG1b proteins are detected at similar (1:1) amounts in native cells (8) we propose that the preference of hERG1b for hERG1a also is a determinant of heteromeric hERG1a/hERG1b subunit assembly in native IKr.

**hERG1a and hERG1b subunits are in close proximity at the plasma membrane, as determined with FRET spectroscopy**

To examine subunit interactions at the plasma membrane, we used FRET spectroscopy. FRET is the transfer of energy from an acceptor molecule (here, enhanced cyan fluorescent protein, eCFP) to a donor molecule (here, monomeric Citrine). Energy transfer is not efficient at distances greater than ~80 Å for GFP-based probes, which makes FRET a sensitive reporter of proximity (24).

We recorded fluorescent images at the cell surface from hERG channels fused to eCFP or Citrine using laser-scanning confocal microscopy (Fig. 5). We measured FRET using the stimulated emission of the donor method (25–29) (see “Experimental procedures”). We co-expressed hERG1b–eCFP and hERG1a–Citrine (Fig. 5A). To determine FRET, cells were excited with a 458-nm laser line and the spectrum was recorded (Fig. 5C, red trace). The component of the spectrum due to eCFP emission was subtracted (Fig. 5C, cyan trace) using a hERG1a–eCFP only spectrum recorded in a separate control experiment, which yielded the subtracted spectrum (Fig. 5C, green trace).
trace). $F_{458}$ was normalized to the emission spectrum from excitation with a 488-laser line ($F_{488}$, black trace), and the ratio of $F_{458}$ to $F_{488}$ (Ratio A, see “Experimental procedures”) contains a FRET component and a component due to direct excitation of Citrine with the 458 laser. To determine the ratio (Ratio $A_0$) due to direct excitation we measured $F_{458}$ (Fig. 5D, red trace) and $F_{488}$ (Fig. 5D, black trace) from a control cell expressing only hERG1a–Citrine (Fig. 5B). The difference between Ratio A and Ratio $A_0$ was greater than zero indicating robust FRET between hERG1b–eCFP and hERG1a–Citrine (Fig. 5E). We also detected robust FRET between hERG1a–eCFP and hERG1b–Citrine (Fig. 5E) indicating that FRET was independent of the donor and acceptor pair. This result shows that hERG1a and hERG1b subunits were in close proximity at the plasma membrane.

In a positive control, we detected FRET between hERG1a–eCFP and hERG1a–Citrine (Fig. 5E) and, although we found that there was weak fluorescence intensity associated with hERG1b–eCFP (see Supplemental Fig. S1) and hERG1b–Citrine at the membrane, which is consistent with the small measurable currents from hERG1b (Fig. 1), we detected FRET between hERG1b–eCFP or hERG1b–Citrine subunits (Fig. 5E). These results showed that hERG1a subunits were in close proximity with hERG1b subunits at the plasma membrane suggesting they were in heteromeric channels.

**Discussion**

Here we report new findings regarding subunit interactions in channels formed from hERG1a and hERG1b subunits. Functional suppression of hERG1a by dominant-negative hERG1b subunits (and vice versa) suggested a direct interaction between hERG1a and hERG1b subunits at the membrane surface (Fig. 2). Disulfide bond formation between hERG1a L589C and hERG1b L249C subunits showed that these subunits were in close proximity (Fig. 3). We detected robust hERG1a L589C–hERG1b L249C dimers when hERG1a L589C and hERG1b L249C subunits were co-expressed, whereas we did not detect hERG1a L589C–hERG1b L249C dimers when subunits were expressed in different cells and co-purified. These results suggested that the hERG1a L589C–hERG1b L249C dimers were from subunits in the same tetrameric channel. We detected FRET between hERG1a–eCFP and hERG1b–Citrine (and hERG1a–Citrine and hERG1b–eCFP) indicating the physical proximity between hERG1a and hERG1b subunits at the plasma membrane (Fig. 5). Our results using different methods show that hERG1a and hERG1b subunits interact at the plasma membrane. We propose that hERG1a and hERG1b subunits form heteromeric hERG1a/hERG1b channels (Fig. 6).

We found evidence that hERG1b prefers to interact with hERG1a. Functional studies showed that increasing the titration of hERG1b relative to hERG1a led to ionic currents with increasingly rapid kinetics of deactivation, but that the kinetics reached a limit or plateau and did not become as fast as kinetics.

**Figure 4. hERG1a–hERG1b dimers with different input ratios of hERG1a and hERG1b.** Shown is a Western blot of: lane 1, hERG1a L589C; lane 2, hERG1b L249C; and lanes 3–5, hERG1a L589C and hERG1b L249C coexpressed in the same cell at the ratio indicated. The same results were obtained in 3 separate experiments.
of homomeric hERG1b channels (Fig. 1). This result can be explained if hERG1b makes a preferred interaction with hERG1a subunits, instead of other hERG1b subunits, and preferentially forms heteromeric hERG1a/hERG1b channels compared with homomeric hERG1b channels. We found biochemical evidence for preferential hERG1b interactions with hERG1a, because upon co-expression of equal amounts of hERG1a L589C and hERG1b L249C subunits we detected robust hERG1a L589C–hERG1b L249C dimers but only weak hERG1b L249C–hERG1b L249C dimers (Fig. 4). Thus, several lines of evidence suggest an assembly mechanism where a hERG1b subunit makes a preferential interaction with a hERG1a subunit.

We detected robust protein expression (and dimer formation) for hERG1b subunits, but we found small current density from hERG1b, and weak fluorescence intensity at the membrane surface from hERG1b–eCFP (supplemental Fig. S1). This result is consistent with the idea that hERG1b subunits make fewer channels at the plasma membrane compared with hERG1a. Our findings are consistent with previous studies showing that hERG1b, but not hERG1a, contains a specific RXR-type of ER retention motif that was partially respon-
Direct hERG1a and hERG1b interactions

The intensity of hERG1b–eCFP in oocytes (31) or hERG1a and hERG1b in HEK293 cells (15) was increased by coexpression with hERG1a subunits at the plasma membrane. We propose that the key factors in determining the make-up of heteromeric hERG channels is 1) the amount of hERG1a and hERG1b and 2) the preference of hERG1b for hERG1a.

Experimental procedures

Electrophysiology

Oocytes were injected with cRNAs and proteins were purified using a modification of a technique previously described (21). Briefly, 4 days post cRNA injection, oocytes (15–25 oocytes per group) were washed twice in 100 mM HEPES, pH 7.6, buffer. Oocytes were subsequently suspended in a buffer containing 100 mM HEPES, 20 mM N-ethylmaleimide in Tris-EDTA (TE), pH 7.6, protease inhibitors and incubated at room temperature for 15 min. Oocytes were homogenized using gentle pipetting, homogenates were centrifuged at 175,000 g for 75 min at 4 °C. The pellets were resuspended in phosphate-buffered saline (PBS) containing 103 mM KCl with protease inhibitors. Material was incubated with 1% SDS at room temperature for 1 h. Samples were loaded and analyzed on non-reducing SDS-polyacrylamide gel. Blots were probed with anti-hERG KA (a gift from Dr. G. Robertson), which recognizes hERG1a and hERG1b, followed by an HRP-linked secondary antibody and visualized with ECL (Supersignal West Femto, Pierce) and an XRS digital gel-imaging system (Bio-Rad).

Biochemistry

Oocytes were injected with cRNAs and proteins were purified using a modification of a technique previously described (21). Briefly, 4 days post cRNA injection, oocytes (15–25 oocytes per group) were washed twice in 100 mM HEPES, pH 7.6, buffer. Oocytes were subsequently suspended in a buffer containing 100 mM HEPES, 20 mM N-ethylmaleimide in Tris-EDTA (TE), pH 7.6, protease inhibitors and incubated at room temperature for 15 min. Oocytes were homogenized using gentle pipetting, homogenates were centrifuged at 175,000 g for 75 min at 4 °C. The pellets were resuspended in phosphate-buffered saline (PBS) containing 103 mM KCl with protease inhibitors. Material was incubated with 1% SDS at room temperature for 1 h. Samples were loaded and analyzed on non-reducing SDS-polyacrylamide gel. Blots were probed with anti-hERG KA (a gift from Dr. G. Robertson), which recognizes hERG1a and hERG1b, followed by an HRP-linked secondary antibody and visualized with ECL (Supersignal West Femto, Pierce) and an XRS digital gel-imaging system (Bio-Rad).
Direct hERG1α and hERG1β interactions

Fluorescence spectroscopy and FRET analysis

FRET was measured using a spectral separation technique (28) that we used in previous studies (25, 26, 29). Oocytes were injected with hERG channel subunits fused to monomeric, enhanced CFP or monomeric Citrine (26, 38). The cRNA ratio of the donor (CFP) to acceptor (Citrine) was 2:1. Fluorescence intensity was measured using a confocal microscope (Leica TCS SP5) at the University of Maryland College Park Imaging Core, Department of Cell Biology and Molecular Genetics. Fluorophores were excited with a 458-nm laser line and emission spectra were measured from 468 to 566 nm (red trace, Fig. 5C). Extracted spectra \( F_{\text{488, green trace}, \text{Fig. 5C}} \) were determined by subtracting scaled spectra from hERG1α–eCFP (cyan trace, Fig. 5C). Cells were also excited with a 488-nm laser line and spectra \( F_{\text{488, black trace}, \text{Fig. 5C}} \) were recorded from 502 to 600 nm. Ratio \( A \) was calculated from the ratio of \( F_{\text{458}} \) to \( F_{\text{488}} \):

\[
\text{Ratio } A = \frac{F_{\text{458}}}{F_{\text{488}}} = \frac{F_{\text{direct}}}{F_{\text{488}}} + \frac{F_{\text{FRET}}}{F_{\text{488}}} \quad \text{(Eq. 1)}
\]

Ratio \( A_0 \) was determined from a control oocyte expressing hERG1α—Citrine, where \( F_{\text{458}} \) is the emission following excitation with a 458-nm laser line and \( F_{\text{488}} \) is the emission following excitation with a 488-laser line.

\[
\text{Ratio } A_0 = \frac{F_{\text{458}}}{F_{\text{488}}} \quad \text{(Eq. 2)}
\]

A value proportional to FRET efficiency, which increases as FRET increases and decreases as FRET decreases is solved by combining Equations 1 and 2.

\[
\text{Ratio } A - \text{Ratio } A_0 = \frac{F_{\text{FRET}}}{F_{\text{488}}} \quad \text{(Eq. 3)}
\]

Author contributions—M. T. designed experiments. B. M., Z. P., and M. T. conducted experiments and analyzed results. M. T. wrote the paper.

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